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**Plants on the move:
Glimpses on cognitive abilities of plants**

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To
my Mom and Dad
(Assunta and Sergio)

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LIST OF ABBREVIATIONS



METHODOLOGICAL TERMS

%	Percentage
1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
95% HDI	95% high-density intervals
ARTT	Analyzer for root tip tracks
ATP	Analysis of root tip tracks
BEST	Bayesian ESTimation supersedes the t test
cm	Centimeters
d	Days
D	Targets differing in distances
Hz	Hertz
ID	Index of difficulty
kHZ	KiloHertz
KLT	Kanade-Lucas-Tomasi
M	Mean
MAD	Median Absolute Deviation
MCMC	Markov chain Monte Carlo method
Mdn	Median
min	Minutes
mm	Millimeters

mm/min	Millimeters/minutes
Mo	Moda
MT	Movement time
N	Total number of the sample
p	p-value
r	Effect's size
W	Targets differing in size
W	W-statistic
x	Sagittal axial
y	Vertical axial
z	z-scores
β	Difference between the mean of the conditions

PLANTS

<i>A. thaliana</i>	<i>Arabidopsis thaliana</i> L.
<i>B. oleracea</i>	<i>Brassica oleracea</i> L.
<i>B. trifoliata</i>	<i>Boquilla trifoliata</i> L.
<i>C. glandulosa</i>	<i>Clamatis glandulosa</i>
<i>C. japonica</i>	<i>Cayratia japonica</i> (Thunb.)
<i>C. pentagonata</i>	<i>Cuscuta pentagonata</i> Engelm
<i>C. viticella</i>	<i>Clematis viticella</i> var. <i>venosa</i>
<i>D. capensis</i>	<i>Drosera capensis</i> L.
<i>D. muscipula</i>	<i>Dionea muscipola</i> Soland. ex Ellis
<i>H. annuus</i>	<i>Helianthus annuus</i> L.
<i>H. helix</i>	<i>Hedera helix</i> L.

<i>Impatiens</i>	<i>Impatiens wallerana</i>
<i>M. pudica</i>	<i>Mimosa pudica</i> L.
<i>O. basilaris</i>	<i>Opuntia basilaris</i> Engelm. & J. M. Bigelow
<i>O. sativa</i>	<i>Oryza sativa</i> L
<i>P. canariensis</i>	<i>Phalaris canariensis</i> L
<i>P. coccineus</i>	<i>Phaseolus coccineus</i> L
<i>P. sativum</i>	<i>Pisum sativum</i> L.
<i>P. vulgaris</i>	<i>Phaseolus vulgaris</i> L.
<i>Passiflora</i>	<i>Passiflora</i> L.
<i>S. jasminoides</i>	<i>Solanum jasminoides</i> Paxton
Tomato plants	<i>Lycopersicon esculentum</i> L.
<i>V. faba</i>	<i>Vicia faba</i> L.
<i>Vitis</i>	<i>Vitis vinifera</i> L.
<i>W. sinensis</i>	<i>Wisteria sinensis</i> (Sims) Sweet
Wheat plants	<i>Triticum aestivum</i>
<i>Z. mays</i>	<i>Zea mays</i> L.

PLANT PHYSIOLOGY TERMS

ACTIN-8	Actins in <i>Arabidopsis thaliana</i> L
AP	Action potentials
ATP	Adenosine triphosphate
BRs	Brassinosteroids
Ca ²⁺	Intracellular calcium
CK	Cytokinin
Co ²	Carbon dioxide

GA	Gibberellins
miRNAs	microRNA
PIN auxin	PIN-mediated auxin transport
RAMOSUS4	Branching regulator RMS4
SLs	Strigolactones
SWP	Slow wave potentials
TIR1/AFB	Transport Inhibitor Response1/Auxin Signaling F-box
VOCs	Volatile chemical compounds

OTHER TERMS

CNS	Central nervous system
CS	Conditioned stimulus
RST	Risk sensitivity theory
SAT	Speed–accuracy trade-off
US	Unconditioned stimulus

SYNOPSIS



Most of us conceive plants as sessile organisms and perceive them as a static colourful background. However, although plants are characterized by a lack of locomotion, they exhibit a variety of movements. If we spend a minute looking at the surrounding, we will notice that plants are in constant motion, and they have evolved several mechanisms to “*cope*” and to “*adapt*” to an ever-changing environment. Many are the cases in which plants reclaim abandoned places creating novel ecosystems. They do this by either modifying their morphology on the basis of different environmental pressures (e.g., urbanization, population, pollution, ...) or elements (e.g., the light, temperature, ...). Both transformations in vegetation space and/or structure are examples of movements in plants. These considerations are at the core of the present thesis, which aims to answer the following questions, are plants able to anticipate and respond according to varying states of their surroundings or simply react passively to environmental elements? Is there evidence of “*goal-directed*” actions in plants? To this end, I used kinematics to characterize the movement of climbing plants movement during the execution of approach-to-grasp movements toward supports with different intrinsic features (e.g., thickness).

The first part of the thesis (Chapter 1) will provide the theoretical framework within which to discuss my data. I shall focus on those theories that assign some forms of cognition to organisms equipped with minimal brains or brainless (i.e., extended and embodied cognition, enactivism; Calvo, Gagliano, Souza & Trewavas, 2020; Calvo & Trewavas, 2021; Parise, Gagliano & Souza, 2020; Segundo-Ortin & Calvo, 2019). The section that follows (Chapter 2) will focus on the different types of movement in plants

with specific reference to the approach-to-grasp movements performed by climbing plants. An overview of the methods and techniques used for studying movement in plants will be also provided.

The thesis then goes on to discuss the experimental work. At the outset of Chapter 3, I shall describe in detail the material and methods, which are common to all experiments. Then in the first experiment (Chapter 4) I shall investigate the approach-to-grasp movement of pea plants (*Pisum sativum* L.; from now on *P. sativum*) in different environmental conditions, namely a condition lacking a potential support (from now on support will be termed as stimulus), either with a stimulus of different thickness (i.e., Thin or Thick) or with the ungraspable pictures of the stimuli [i.e., two-dimensional (2D) Thin or Thick stimulus]. This permitted to investigate whether plants can perceive an element in the environment and to plan a movement based on the structural properties of such element. In Chapters 5 and 6, further experiments aimed to assess if and how *P. sativum* plants modulate movement velocity strategically depending on the difficulty of the task. More specifically, the experiment described in Chapter 5 was set to investigate whether *P. sativum* plants can scale movement velocity as a function of the difficulty to coil a stimulus, obeying to the Speed–Accuracy Trade-off (SAT) phenomenon, the tendency for movement speed to covary with movement accuracy. A further experiment (Chapter 6) investigated if *P. sativum* plants evolved a motor accuracy mechanism as to improve the precision of their movement. To this end, I investigated whether *P. sativum* plants could correct online their movement by means of secondary movements (i.e., submovements), and if their frequency production is influenced by the difficulty of the task (Chapter 6). Altogether these experiments showed that *P. sativum* plants can plan a movement based on different stimulus properties and provided preliminary evidence of “goal-directed” movement in plants. Indeed, *P. sativum* plants not only acknowledged the presence of a

stimulus in the environment, but also scaled the kinematics of their tendrils (i.e., long and filiform organs used by the plant to grasp a stimulus) in term of velocity and aperture (i.e., the distance between the tips of the tendrils) based on different stimulus thickness and as a function of the difficulty to coil it. In Chapters 7 and 8 I shall report on two experiments aiming at revealing the possible mechanisms underlying the processing of stimulus thickness. These experiments explored the possible contribution of the above- (e.g., tendrils, apex, ...) and the belowground (i.e., the root system) organs of plants in stimulus thickness coding process. In particular, for the experiment reported in Chapter 7 a stimulus was either inserted in or lifted to the ground so that the stimulus information was available (or not) to the root system. The results indicated that when the stimulus was not available to the root system, plants were not able to locate it in the surrounding and to modulate the pattern of their approaching and grasping movement with respect to stimulus thickness. Thus, these results suggest the possible role of the root system in the coding of stimulus thickness. To better assess this aspect, in the experiment reported in Chapter 8, the below- and aboveground thickness part of the stimulus was varied: in one condition the belowground part of the stimulus was thick, whereas the above part of the stimulus was thin. In another condition the opposite combination was tested. Control conditions in which a one-sized stimulus (i.e., Thin or Thick) was presented to the plant, were compared to the perturbed conditions. Results not only confirm the contribution of the root system in sensing, coding, and processing belowground information, but also that such information is evaluated and eventually modified at the level of the aerial part of the plant to fulfil the end-goal of the movement. Results are discussed in terms of a functional equilibrium reached through a crosstalk between the grounded and the aerial components of the plant.

Finally in Chapter 9, a general discussion outlining the theoretical implications of these findings will be provided. Importantly, my results provide preliminary evidence of

“goal-directed” actions in plants and suggest the existence of a process that may link perception to action in organisms without a brain or a central nervous system (CNS). These results pave the way for the comprehension of the mechanisms showed by brainless organism for adapting their behavior to an ever-changing environment and provide new insights regarding the evolution of the link between cognition and action.

PART I



THEORETICAL BACKGROUND

CHAPTER 1



EXTENDING COGNITION

The purpose of this chapter is to provide a possible theoretical framework within which to discuss the experimental results of the present thesis, suggesting that plants explore the environment to meet their own needs and goals, instead of simply reacting to the external impingements. And that they are capable of actively regulating their sensorimotor coupling in context-sensitive ways. Overall, I aim to motivate the idea that plants may be considered genuine cognitive agents as far as their motor behavior is concerned. In this perspective, plants appear to behave in ways that are adaptive, flexible, anticipatory and “*goal-directed*” (Calvo, 2017; Castiello, 2020; Gianoli, 2015; Guerra & Castiello, 2021; Parise, Reissig, Basso, Senko, Oliveira, de Toledo et al., 2021; Raja, Silva, Holghoomi & Calvo, 2020; Trewavas, 2009; 2014; 2017; Wang, Guerra, Ceccarini, Bonato & Castiello, 2021). Taking this into account, I shall argue that plant behavior is in many ways analogous to animal behavior, meaning that plants are suitable candidates to be described as cognitive agents in a non-metaphorical way.

1.1. What is cognition?

The term cognition commonly refers to “*all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered and used*” (Neisser, 1967).

Cognitive functioning is fundamental for daily life activities given that it organizes thoughts and actions by helping individuals to assign a meaning to the world’s experiences, understand them and respond to them properly. There are many types of cognitive processes such as: (i) attention, which allows individuals to focus on a specific

stimulus or situation in the environment; (ii) language, which involves the ability to understand and express thoughts by means of the spoken or written words in order to communicate to other individuals; (iii) memory, which permits individuals to encode, store and recall the information and knowledge about past experiences and the world; (iv) perception, which is used to take environmental signals through the senses (e.g., smell, touch, ...) and then use them to respond and interact with the world, properly; (v) thought, which allows individuals to engage in decision-making, problem-solving, and higher reasoning. All these capacities allow individuals to enrich their repertoire of behavioral responses which are used to solve problems and cope with environmental difficulties in the short term and/or in the nearby future. An important aspect of cognition is its flexibility, which leads individuals to constantly change and adapt themselves to newly information and conditions across their lifespan. Since infancy to later adult life, cognitive functioning is constantly developing by integrating both environmental and genetic factors allowing individuals to produce and regulate their behavior depending on different environmental conditions.

The classical view of cognition assumes that cognitive capacities are brain-based and localized in specialized brain regions (e.g., the language in the Broca's area). In this view, the brain has the function to fill the gap between the external world as it is and the sensation (i.e., retinal image, sensory stimuli, ...) that the agent extracts from it by means of his/her/its perceptual and sensory systems. In other words, environmental inputs are sensed through the perceptual process and then transmitted to the brain, which processes and manipulates them into internal representations (i.e., concept and ideas about the external world). Indeed, given that the environmental stimulations are poor of contents *per se*, an internal enrichment and disambiguation of the sensory data by means of the development of an image of the external world in the brain seems to be needed (Fodor &

Pylyshyn, 1981; Marr, 1982; Ullman, 1980). Then, mental representations are operationalized by means of the motor system that implements behavioral responses accordingly.

To summarize, all the sensations that we experience from the surrounding through vision, smell, taste, hearing, and touch became signals that are transformed in impressions and beliefs by the brain and then used to understand, judge, and solve daily life events. The brain, thus, could be defined as the place in which beliefs, thoughts, emotions, and actions are generated and developed, and it is where our understanding of others and the world take shape. Echoing the words of Francis Crick “*You’re nothing but a pack of neurons*” (Crick, 1996; Crick & Clark, 1994). But are we and our behavior just a result of “*a pack of neurons*” or there is more? Along the classical view, the brain has the principal role to generate cognitive processes, while the body and the environment seem to stay in the background. Furthermore, the notion of cognition is commonly associated to an information processing mechanism that describes how the human mind operates in the world. This perspective led to assign cognitive capabilities, such as problem solving and intentionality, only to human beings leaving out other organisms whose behavior does not mirror human-style-reasoning.

For over fifty years philosophers have been re-thinking of the nature of cognition, moving the attention from the central role of the mind to the agent, the environment, and their interaction. Post-cognitivist perspectives assume that cognition would take place when the organism is inserted in the ever-changing real world, and it involves perception and action. In other words, the environment affects the organism, and the degree to which the environment produces an effect on the agent depends on the organism’s ability to perceive external signals and to organize them freely and autonomously (Cazalis, Carletti & Cottam, 2017; Gomila & Calvo, 2008). In this perspective, cognitive control is extended

to all parts of the body and to physical objects in the world. An example is given by bacteria. Bacteria can monitor and assess the significance of the signal they receive from their environment by means of a variety of sensory systems, integrate them with internal signals (e.g., intracellular physiological status) and adapt their behavioral responses to ensure their survival (Lyon, 2015). Therefore, bacteria seem to be able to control and adjust their own behavior to interact and cope with different environmental situations. The absence of a brain and a CNS does not impede bacteria to have and experience a sort of “*cognition*”. But could the term cognition be extended to brainless organisms such as plants?

Classical theories in cognitive science assume that cognition arises from a unidirectional process concerning “*Perception*” » “*Planning*” » “*Action execution*”. In this view, perception allows the agent to build an internal representation of the surrounding (Marr, 1982). Recently, alternative theories termed as action-oriented paradigms (e.g., extended cognition and enactivism) state that cognition is not just “*in the head*” but it concerns the environment, the agent’s body, and the external object (Dewey, 1896; 1916; 1938; Peirce, 1887). That is, cognitive processes are conceived as deeply rooted in the embodied agent’s interactions with the world (Clark, 2013; Clark & Chalmers, 1998; Varela, Thompson, & Rosch 1991; Wilson, 2002). The main aim of theories such as enactivism (Thompson, 2007; 2016), extended (Clark & Chalmers, 1998) and embodied cognition (Keijzer, 2017; Varela et al., 1991) is to provide a new meaning for cognition that may fill the gap between the mindful and the mindless considering brainless organisms as cognitive agents. Below I shall provide an overview of these theories, and how they may explain the behavior of plants.

1.2. Cognition without a brain

Taking over the definition of cognition by Neisser (1967) “*all processes by which the sensory input is transformed, reduced, elaborated, stored, recovered and used*”, it becomes evident that it could be applied to other organisms than human beings. Since the late 1990s, the classical view of cognition - holding that it takes places in the brain after perception (i.e., acquiring information from the environment) and before motor processing (i.e., signals are converted in concepts which become motor behavior) - has been challenged. And a new concept implying cognition as an emergent and extended self-organized phenomenon stemming from the interaction between an organism and the environment has been advanced. This view has paved the way for including in the cognitive domain other type of organisms with no brain or CNS showing complex behaviors as to cope with different environmental challenges to ensure their survival (Calvo, 2007; Trewavas, 2005; 2009; 2014).

Let’s consider the case of plants, which is, of course, relevant for the present thesis. Plants can sense a wide range of environmental signals such as light, gravity, soil nutrient and the presence of neighbors by exploiting a variety of behavioral responses (Calvo & Keijzer, 2009; Trewavas, 2017). Plants can “*smell*” the volatile chemical compounds (i.e., VOCs; e.g., benzene, ethylene glycol, ...) emitted by other plants, which are attacked by insects or predators and prepare an appropriate defense for possible assaults (Karban, 2015; Karban, Shiojiri & Ishizaki, 2011; Karban, Wetzel, Shiojiri, Ishizaki, Ramirez & Blande, 2014). Plants can sense the presence of food resources and forage them by increasing or reducing the morphology and physiology of their roots or shoots depending on the abundance of the perceived resources (de Kroon & Hutchings, 1995; Grime & Mackey, 2002; Hutchings & de Kroon, 1994). Plants can also learn and remember as to react more effectively to future challenges. Indeed, memory for non-fatal

attack remains latent in every cell of the plant, and it is expressed only at the time that a similar experience occurs again (Hammerschmidt & Kúć, 1995; van Loon, de Boer & Dicke, 2000). In another example learning was investigated by means of the classic conditioning paradigm of Ivan Pavlov (1927). This paradigm is characterized by the sequential presentation of a conditioned stimulus (CS), which is generally a neutral stimulus and biologically not important for the subject (e.g., sound of a bell), and an unconditioned stimulus (US; taste of a food) which elicits a response in the subject automatically (e.g., salivation). After many presentations of both stimuli together, the neutral (or conditioned) stimulus leads to an automatic response when it is presented alone. Therefore, the subject acquired through experience a new type of behavioral response. The same paradigm was administered to *P. sativum* plants, which were tested by means of two different stimuli, a light source (i.e., unconditioned stimulus) and an air flow (i.e., neutral, or conditioned stimulus). Specifically, the presentation of the air flow indicated to the plant when the light would appear (Gagliano, Vyazovskiy, Borbély, Grimonprez & Depczynski, 2016). Results showed that the *P. sativum* seedlings grew toward the air flow even though the light source was not present. This suggests that *P. sativum* plants developed an association between the light source and the air flow, thus learning a new behavior (Gagliano et al., 2016).

Moreover, it has been demonstrated that plants can sense the level and the variability in resources in the environment and evaluate the success and failure rates of the possible scenarios based on the provided information switching from a state of propensity and aversion to risk (i.e., the risk sensitivity theory – RST; McNamara & Houston, 1992). Dener, Kacelnik and Shemesh (2016) divided the roots of *P. sativum* plants in two different pots with different level of nutrient concentrations, which could be either constant or variable. Results showed that in presence of constant nutrient concentrations *P.*

sativum plants grew more roots in the pot with higher level of nutrients. While, when the level of nutrients in the pot with constant concentration was not enough for the plant to survive, they allocated more biomass in the pot with a variable nutrient concentration. These results suggest that the plants can sense and respond properly to the varying of nutrient availability in the soil and therefore able to switch between risk-prone and risk-averse behavior as a function of resource availability in a similar manner as observed in different animal species (e.g., social insects, birds, primates, ...) including human beings (Dener et al., 2016; Kacelnik & Bateson, 1997).

All these examples suggest that plants show a variety of behaviors and strategies to cope with different situations, which are not a simple automatic and fixed reaction to the environment. In the light of such evidence how can the behavior of plants be defined? Can plants be described as cognitive agents? In the next sections I will consider the possibility to extend the term cognition to brainless organisms such as plants by capitalizing on recent theories, namely embodied, extended cognition, and enactivism.

1.2.1. Embodied cognition

The predominant view about cognition (i.e., cognitivism) considers the body of the agent as peripheral in the development of cognitive processes. Newly embodied cognitive sciences have, instead, suggested new ways to explore and conceptualize cognition (Keijzer, 2017). In this view, cognition is shaped by the entire aspects of the body of the agent, which senses and acts in the reality of the world, rather than being the product of a brain developing innate abstract representations of the environment. By following the embodiment thesis, the body of the agent can be considered as a distributor for cognitive processing, or as a regulator of cognitive activity. Thus, the body of an agent, which is considered as a dynamic instrument of exploration, may have the role to organize and

regulate cognitive activities over time and space ensuring the functional coordination between cognitive processes and action. In this view, cognitive representations of the external world (e.g., beliefs, desires, perceptions, ...) are “*sublimations*” of the bodily experiences instead of being established by the mind autonomously (Merleau-Ponty, 1945; 1963). Therefore, cognition is distributed among the whole body of the agent rather than solely in the brain, which is not the unique resource used by an organism to solve problems. In sum, the embodied cognition thesis could be summarized in three main concepts: (i) the brain seems to have a marginal and minimal role in cognition; (ii) body structures of the agent play an important role in the development of the cognitive processes and in organizing the cognitive activities; (iii) cognition depends on the type of experience that the body of the agent senses and comes across with different sensorimotor capabilities. With this in mind, it is not too hard to conceive brainless organisms as cognitive agents. However, it is important to delineate the main five constraints characterizing embodied cognition so to assess whether such theorizing can be extended to plants. First, being a cognitive agent implies having a guiding principle which drives behavior and helps the agent to discriminate between relevant and irrelevant and/or convenient from the inconvenient environmental stimuli. In this view, the metabolism (i.e., the set of life-sustaining chemical reactions in organisms) provides a basic and normative cognitive criterion which permits the agent to discriminate across different stimuli and conditions which of them are “*bad*” or “*good*” for the organism (Bickhard, 2008). Second, a cognitive agent should be able to use the relevant information of the environment to ensure its survival and enhance its fitness. Third, the agent can access to the structures of the environment by means of its free-bodily movement and to sense them by means of its sensorimotor organization. Fourth, the sensorimotor organization allows the agent to perceive the environmental stimuli and activate a motor response toward them

but also to modulate and adapt its behavior depending on the generated outcomes. Fifth, a cognitive agent can store the information provided by the relation between the individual stimulus–response and organize it into a coherent unit, which may be used by the agent to respond to future events, properly.

Regarding the first two concepts, there is no doubt that they can be applied to plants. Indeed, plants can sense and code a wide range of environmental elements and they can modulate their growth and foraging behavior accordingly. For instance, plants alter the spatial distribution and the morphology of their roots as a function of resource patchiness (e.g., soil heterogeneity, different level of soil nutrient, ...) or they increase or decrease their stem and root growth depending on the presence of neighboring plants and their kinship (i.e., kin or stranger relationship; Cahill, McNickle, Haag, Lamb, Nyanumba & Clair, 2010; Cahill & McNickle, 2011; Dudley & File, 2007; McNickle, Clair & Cahill, 2009). The real issue for extending cognition in plants comes from the remaining concepts. The third concept assumes that a necessary requirement for cognition is that of being a free-moving organism with a sensorimotor organization. In general, behavior is commonly restricted to actions involving movements. Taking in mind this assumption, it would be difficult to consider plants as cognitive agents given that they are considered as sessile organisms without visible movements. However, this is a false belief because plants move a lot, and they do it in a fashionable manner (as the work in this thesis will further demonstrate). Some movements are visible in real time such as the closure of the leaves of the *Mimosa pudica* L. when they are touched by an external stimulus considered by the plant as dangerous (e.g., the human finger). Other types of movements are difficult to perceive in the reality given that plants move in a different timescale from the animal modality. However, by using specific time-lapse recording techniques movements of plants can be perceived and appreciated by us. For instance, we can observe the

germination of the seeds or the blooming of the flowers. The important gap between the animal and plant motion is that animals move from one place to another to avoid predators as well as to find and gain food resources, whereas plants are anchored to the ground. However, plants display other types of movement concerning the elongation and the modification of the organs which allow them to explore the environment (Darwin & Darwin, 1880). Then, every single organ of plants can perceive and code environmental signals and put in place a suitable response. In this view, plants might be considered as “*free-moving organism with a sensorimotor organization*”. Then, the fourth and fifth concepts highlight that the basic sensorimotor organization operates both online and offline involving a control of the system. These two points state that organisms can sense and elaborate the external signals, regulate their behavior by integrating novel information with previous ones and act on the environmental elements to change conditions therein or their own situation in relation to their surroundings. This assumption implies a form of “*motor intentions*” in the terms of “*goal-directed*” behavior (Wang et al., 2021). In general, an action is defined as intentional or “*goal-directed*” when it is tuned to the task and its execution is under voluntary control of the agent. According to this definition, the main goal of the action persists in the agent’s phenomenological experience throughout the time the action is unfolding and until it has been completed.

But could the term “*motor intentionally*” refer to the movement of plants? In other words, are plants able to act “*intentionally*”? According to the classical view of cognition, the development, and the manipulation of the metabolic representation of the surrounding is a fundamental process to act and behave cognitively in order to act intentionally plants should be able to sense and process the external stimulation, transmit these sensory inputs to a central system - like the CNS - to process and translate them in concepts or intentions (i.e., mental representations) and then operationalize them into suitable motor responses

and behaviors (Souza, Toledo & Saraiva, 2018). In this view, clearly plants might not be equipped to act intentionally. However, following the embodied theory cognitive capabilities do not necessarily depend on mental representations, but they are developed from the interaction of the bodily structures of the agent and the environment - the response is “*Yes*” (Clark, 2008; Gallagher, 2005). Indeed, plants can actively explore the environment, perceive, and pick up information from the elements in it without using internal representations or processing, and combine them with internal information concerned with their internal state (Baluška, Lev-Yadun & Mancuso, 2010; Baluška, Mancuso, Volkmann & Barlow, 2004; 2009; Batenson, 1985; Calvo & Trewavas, 2021; Maturana & Varela, 1980; Trewavas, 2005; 2007; 2014; 2016; 2017). Then, plants are able to use the environmental stimulations, which are rich of information, to control and coordinate their behaviors and actions. The central point is that in plants, intentionality is expressed by each part of its body (i.e., shoot and the root system) that produces appropriate and functional responses.

In sum, behavior of plants seems not to be a merely collection of automatic stimulus-response but a globally organized cohering unit. That is, cognitive abilities seem not to be localized in single unit as the brain, but rather in each organ of the plant. In this view, plants could be defined as “*cognitive*” in the general sense proposed by the embodied cognition theory.

1.2.2. Extended cognition

Extended cognition theory holds that the brain-body-world are dynamically coupled, and thus the environment is considered more than a simple background for the cognitive system but a necessary part of it (Clark & Chalmers, 1998). In other words, cognitive capabilities arise from the dynamic and mutual interaction between the body of

the agent and the external world, which affects each other reciprocally (Gibson, 1979). Indeed, the dynamical interaction between the agent and the environment allows the organism to pick up different environmental information, which became available thanks to the active exploration of the surrounding. The organisms, in turn, use such information and the resources (i.e., affordance) that an environment offers to control and coordinate their behavioral responses toward the external world. However, the resulting behavior can be influenced by the presence of environmental fluctuations (e.g., water deficit, different level of temperature, ...). The level at which the behavior is affected depends on the ability of the organism to perceive the external signals and maintain its auto-organization (Souza & Lüttge, 2015; Souza, Ribeirrom, Prado, Damineli, Sato & Oliveira, 2009; Yates, 1983; 2012). Further, differences in the selective perception of environmental signals across organisms determine what is available to them. For instance, plants can sense and detect the VOCs emitted by neighboring plants or insects, while human beings cannot because they have a different perceptual system, and thus that environmental information is irrelevant for them. In other words, environmental information is meaningful for an organism, and not meaningful *per se* (Gibson, 1979; Segundo-Ortin, Heras-Escribano & Raja, 2019).

Therefore, the formation of the cognitive processes arises from both internal (i.e., the individual's ability to use the relevant structures in its environment) and external (i.e., the manipulation, exploitation, and transformation of environmental elements) processes (Craver, 2007; Kaplan, 2012). Let's consider the example deriving from the relationship between the spider and its web (Japyassú & Laland, 2017; Parise et al., 2020). The different form of vibrations from the web generated by an external element such as insects produce a kind of information that is used by the spider to improve its foraging behavior strategy. For instance, the spider may manipulate the tensional force of the web threads to

enhance its possibility to catch more insects. In this case, the dynamic and mutual relationship between the spider and its web affects and modulates the cognitive capacities of the spider leading to the comprehension of the surrounding (Japyassú & Laland, 2017; Nakata, 2010; 2013). But could the extended cognition theory be applied to plants? Given that plants are grounded in the soil, and they cannot move from one place to another acting actively on the external object, how can they extend their cognition?

Two possible ways in which plants might extend their cognition to the world have been advanced: (i) the root exudates and (ii) the relationship between the root system and the mycorrhizal fungi. In first instance, the root exudates are substances secreted by the roots in the rhizosphere (i.e., the part of the soil around the root system) which play an important role in mediating both positive (e.g., symbiotic associations with beneficial microbes such as mycorrhizae) and negative interaction (e.g., parasitic plants, herbivores, ...) between neighboring plants and the microbes (Bais, Park, Weir, Callaway & Vivanco, 2004; Bais, Weir, Perry, Gilroy & Vivanco, 2006; Broeckling, Broz, Bergelson, Manter & Vivanco, 2008; Weir, Park & Vivanco 2004). The relationship between the plant and its root exudates, which may be considered as the relationship between a spider and its web, might constitute the plant's cognitive system. To clarify this aspect, let's consider the avoidance response behavior of the plant's roots (Falik, Reides, Gersani & Novoplansky, 2005). That is, the ability of plants to modulate the growth direction and the morphology of the roots depending on the presence of obstacles in the soil. The plant's ability to circumvent physical obstacles is necessary to move freely in the soil so to gather the nutrient necessary to ensure its survival, especially in rocky environment. Charles and Francis Darwin (1880) observed that the tip of *Vicia faba* L. roots can modify its morphology and growth direction after encountering a thin glass plate at a steep angle. Since Darwin's observations, Wilson (1967) reported that the woody roots of the maple

trees can sense the presence of barriers into the ground and rapidly change their growth direction far away from the detected obstacles. The root exudates are a likely candidate for this behavior. Indeed, it has been demonstrated that the accumulation of the root exudates between the obstacle and the root cap may allow plants to perceive the object in the soil and to inhibit root growth toward it (Falik et al., 2005; Semchenko, Zobel, Heinemeyer & Hutchings, 2008). Then, the removal of the root exudates from the belowground surrounding affects the plant's ability to perceive and localize the object in the soil (Falik et al., 2005). Therefore, the root exudates might act as a dynamic link between the plant and the external world transmitting crucial information, which are used by the plant to shape its internal states and to produce functional behavioral responses toward the surrounding.

In second instance, plant can extend their cognitive capabilities by means of their association with the mycorrhizal fungi. A connection between the root system and fungi or bacteria in the soil which led the constant transmission of different molecule, nutrients, and signals among them. Signals generated by the mycorrhizal fungi are used by the plant to extend its perceptual system to gain information from areas outside the plant's reach. Therefore, the fungal network seems to be a likely candidate to extend the plant's perception of the environment and to facilitate the forming of cognitive processes in plants.

Another example of extended cognition in plants is provided by the emission of the VOCs, which plays a crucial role in plants interactions with biotic (e.g., bacteria, animals, ...) and abiotic factors (e.g., water, sunlight, ...). The emission of the VOCs in the air is used by the plants to activate a variety of behaviors (Vivaldo, Masi, Taiti, Caldarelli & Mancuso, 2017). For instance, a defense mechanism towards insects or other predators (e.g., Mumm, Schrank, Wegener, Schulz & Hilker, 2003), pollinators attraction (e.g.,

Dudareva & Pichersky, 2000) and communicating with plants in the nearby (e.g., Bonato, Peressotti, Guerra, Wang & Castiello, 2021; Heil & Karban, 2010). For instance, when the plant is attacked by an insect or an herbivore it starts to release VOCs in the surrounding in response to the insult. Then, VOCs are intercepted by the self-plant's organs or neighbor plants leading to the activation of a variety of defense mechanisms which helps them to be prepared for a forthcoming attack (Baldwin, Halitschke, Paschold, von Dahl & Preston, 2006; Farmer & Ryan, 1990; Karban, Huntzinger, & McCall, 2004). In this case, the relationship between the plant and the external organism (e.g., the insects, the herbivore, ...) causing a damage for the plant's fitness through the transmission-reception-sensing strategy produces information useful to modulate and improve the plant's defense strategies. In sum, the above evidence suggests that plants can incorporate the physical elements of the surrounding in their cognitive system and extend their cognition beyond their bodies (Parise et al., 2020).

1.2.3. Enactivism

Beside the extended cognition theory there is enactivism, which capitalizes on the idea of sensorimotor contingencies, that is perception is shaped by sensory stimuli and by the actions of the organism. In detail, the sensorimotor contingency theory predicts that action and perception are linked together in a bidirectional relationship in which they influence and change each other (O'Regan & Noë, 2001; Varela, 1992). The enactivism approach was formulated as a rejection of the information processing and symbolic representations concepts that dominate cognitive science (Thompson, 2007). Indeed, one criticism to the cognitivist theory is the lack of interest about the role of the bodily agent, its subjective experience, the environment, and their dynamic interaction in the development of cognitive processes (Thompson, 2016). In this view, enactivism sets itself

as a radical change in understanding the mind and life in cognitive science. This theory focuses its attention on the dynamic interaction between an acting organism and its environment which has a central role in the development of cognition (Hurley, 1998; Noë, 2006; Varela et al., 1991). The interaction between the agent and the world, which contains the information useful for guiding and specifying the agent's behavior, is necessary for the development of cognitive states which are not sole brain-bounded (Chemero, 2013). Enactivism stresses the importance of the body of the agent in the development of cognition considering it not only as a center of experience, but also as a center of agency (i.e., the ability to act actively on the reality). The body is concerned as the vehicle for experiencing and understanding the surrounding and for driving the perceptual system in the exploration of external world by acquiring the meaning of environmental stimuli through motor responses and behaviors (Di Paolo Rohde & De Jaegher, 2010; Engel, 2010; Engel, Maye, Kurthen, & König, 2013; McGann, 2007; Varela, 1992). In this view, cognition is considered as the capability of an organisms to actively interact with the environment in an adaptive, flexible, and sophisticated manner to maintain its systematic autonomy. Thus, the only way to explain cognitive capabilities of a given organism is to consider its environment. A cognitive system is thus conceived as an autonomous and open system which led the organism to explore its surrounding and to interact with it satisfying its needs. More specifically, the cognitive system is considered as capable to actively regulate the agent's responses toward the environmental stimulation instead of simply reacting to them to meet her/his/its goals (Segundo-Ortin & Calvo, 2019).

As previously reported, a growing number of evidence has demonstrated that plants can control their behavior and respond to the surrounding properly (Trevawas, 2005; 2009; 2017). Plants can “*record*” and “*remember*” their states of affairs moments

ago and use this information to make a decision in its current state (Gagliano et al., 2016; Proffitt, Khallaf, Carrasco, Larsson & Anderson, 2015). Plants can also perceive the presence of neighbors, interact with them by receiving and emitting chemical substances (i.e., VOCs, root exudates, ...) in the air or/and in the soil (Karban, 2008; Karban & Shiojiri, 2009; Karban et al., 2014; Dudley & File, 2007; Dudley, Murphy & File, 2013) and to modulate their behavior depending on perceived signals (Karban et al. 2004; 2006; Shiojiri & Karban 2006). Moreover, plants can explore their surrounding and actively regulate their behavior depending on different environmental conditions (Gianoli, 2015; Parise et al., 2021; Runyon, Mescher & De Moraes, 2006). It has been demonstrated that climbing plants, which need to find a potential support to grow vertically and reach the greatest source of light, can sense the properties of the support (e.g., roughness), and make decision based on these information (Darwin, 1875; Gianoli, 2015; Carrasco-Urra & Gianoli, 2009; Rowe, Isnard, Gallenmüller & Speck, 2006; Tronchet, 1945; 1946; 1977). Ecological studies carried out in forest showed that the amount of vine climbers is reduced with an increase of the trunk diameter given that it is perceived as unsuitable, thus affecting the success of vines in reaching the upper layers of the forest to enhance light capture (Carsten, Juola, Male & Cherry, 2002; Gianoli, 2001; 2003; Putz, 1984; Putz & Holbrook, 1992). In this case, climbing plants explore the surrounding, anticipate what is going to happen and “*make decisions*” freely based on the information provided from the surrounding (Severino, 2021). They regulate their behavior to enhance their chances to satisfy their endogenous needs (e.g., reach the light source). Another example is provided by the *Passiflora* (*Passiflora* L.) which can modify the movement of its tendrils depending on the different position of the support. That is, even though the support was switched to a different position the tendrils of the *Passiflora* continued to change the direction of their movement as to approach and grasp it (Baillaud, 1962).

These examples show that plants can sense the properties of a potential support in the environment and to discriminate between adaptive or maladaptive environmental elements. Thus, plants seem to be sensitive and reactive to different elements or resources that the environment offers by modulating their behavior and actions accordingly.

CHAPTER 2



PLANTS IN MOTION

2.1. On the characterization of plant movements: an overview

“It has often been vaguely asserted that plants are distinguished from animals by not having the power of movement. It should rather be said that plants acquire and display this power only when it is of some advantage to them ...”

– Darwin, 1875 –

Since plants are unable to move from one place to another, they are commonly conceived as still organisms. However, even if plants are characterized by a lack of locomotion this does not mean that they show any movement. Plants may not move as far or as quickly as animals, but they are hardly immobile. The important issue here is that some plants’ movements achieve many of the same functional ends as those of animals (Huey, Carlson, Crozier, Frazier, Hamilton, et al., 2002). For instance, a part of the life cycle of many plants and animals implies relocation to a new site (Croteau, 2010). A common form for such relocation is dispersal, an ecological process that involves the movement of an individual (or multiple individuals) away from the population in which they were born to another location, or population, where they will settle and reproduce (Croteau, 2010). Dispersal can be active or passive. The former is common in both adult and juvenile animals and involves movement of the entire organism through its own ability. Passive dispersal involves both plants and animals unable to move that uses dispersal units called disseminules to aid in reproduction or the exploitation of new

environments (Croteau, 2010). Many disseminules are adapted for movement by specific dispersal agents like wind, water, or another animal capable of active dispersal. Among the sessile adult animals that utilize passive dispersal there are invertebrates like sponges and corals. Their disseminules are typically specialized buds or cells used in reproduction. In plants, seeds, spores, and fruits are the most common disseminules (Sorensen, 1986; Willson & Traveset, 2000). All of them have modifications for movement away from the parent plant via available environmental kinetic energy. Some disseminules are explosively released over short distances whereas others fall to the ground at the base of the parent plant. Seeds and fruits are scattered by invertebrates, mammals, and birds during feeding and distributed in feces after ingestion. Water currents, winds and flying animals are amongst the most successful agents of long-distance passive dispersal. Seeds and fruits that have wings, hairs, or inflated processes are carried efficiently by wind. In addition, some plants have sticky seeds, or fruits that adhere to the feathers or fur of mobile animals (Croteau, 2010; Sorensen, 1986; Willson & Traveset, 2000).

Some plants have responses that achieve much the same ends as the locomotor adjustments of animals. Plants can effectively choose where to live by growing towards the needed resources or away from environmental stressors (Bazzaz, 1991). Neotropical stilt palms (*Socratea exorrhiza*) move on their stilts towards light gaps (Leopold, Jaffe, Brokaw & Goebel, 2000). Others such as climbing and clonal plants can crawl across the environment in search of appropriate habitats as animals do. In fact, such movements are sometimes called “*foraging*” (Harper, 1977).

The difficulty to perceive movement in plants is given by the fact that plants operate in a different time scale from animals, and this has made plants to be considered as passive organisms, which are unable to interact with their surroundings. This idea was challenged by Charles Darwin (1875; 1880) who was one of the first interested in studying

movement in plants. One of the great advantages of Darwin was his no botanical background, which let him to approach plants with “*new eyes*” and with any preconceived bias. At first, Darwin was interested in studying the carnivorous plants such as the *Drosera* (*Drosera capensis* L.) and the Venus Flytrap (*Dionea muscipola* Soland. ex Ellis). Darwin focused his attention on the rapid and strong movement of the *D. muscipula*, a plant which is capable to trap insects (e.g., flies) within its leaves, that once ingested produces nitrogen a fundamental element for plant’s life.

Afterwards, Darwin approached climbing plants. In 1875 he wrote a book entitled “*The movements and habits of climbing plants*” in which he described in detail the revolving movement of the stem and the tendrils (i.e., modified leaves that allow plants to climb a potential support) among various classes of climbing plants (i.e., twining plants, leaf-climbers, tendril-Baerers and root climbers) and the purpose of that behavior. Indeed, he was fascinated by the ability of climbing plants to transform and to adapt their behavior to ensure survival. For instance, how climbing plant modify their pattern of movement to reach and coil around a potential support and to respond to different environmental stimuli (e.g., light, gravity, ...).

In 1880, Charles Darwin and his son Francis published a book entitled “*The Power of movement in plants*” in which they described how they examined in detail all types of movements of plants by drawing the trajectories of the tip of shoots through time and space. Plants growing in a pot were covered with a horizontal and a vertical sheet of glass upon which movements of shoots were reported by means of dots every 1-2 minutes. The discoveries of Darwin contributed to shed light on the ability of plants to perceive, to sense, and to response to the environment properly and in an adaptive way. Darwin identified two major classes of movements: tropism, a movement in response to an external factor (e.g., the light) and nastic movement, which depends to external elements,

but its direction is independent with respect to the stimulus position (e.g., closure of the leaf during the dark period; Migliaccio, Tassone & Fortunati, 2013). Furthermore, he reported that all parts of the plant (e.g., stem, tendrils, ...), even if often on a smaller scale, are in constant motion and they tend to rotate around their central axis during the growing phase. This pattern of movement, which was at first named by Julius von Sachs as “*rotating nutation*” and then renamed as circumnutation by Charles Darwin, was defined as common and universal among all plants (Darwin, 1875; Darwin & Darwin, 1880). Furthermore, Darwin was also impressed by the power of movement of the root’s tip, claiming that it may be able to receive information from the other sense organs and direct the movements of the other parts of the plant, “*acting like the brain of one of the lower animals*” (Darwin & Darwin, 1880).

2.2. Types of movement in plants

If I ask to anyone the main difference between animals and plants the simple and natural answer is that plants do not move. This is a false belief because plants move a lot and in a fashionable manner (Darwin & Darwin, 1880; Trewavas, 2014; Wang et al., 2021). Commonly, movement refers to locomotion, that is the moving from one place to another, and it is attributed only to animal species, including human beings. However, controlled locomotion is also observed in some organisms such as flagellates and algae. But in most cases plant’s movements occur as slow and tiny changes in orientation and size in various organs of plants. Movements is used by plants to deal with a wide range of problems such as finding and reaching an environment that provides adequate level of nutrients or protecting themselves against severe climate changes. In general, movement in plants concerns the elongation or the shortening of organs. Underlying this mechanism there are four distinct types of processes: turgor, growth, hygroscoy and drying. Turgor

refers to changes in size of specific cells which determine the movement of leaf blade or a reproductive structure. Growth concerns changes in size, form, and orientation of the whole plant's structure during its life. The asymmetrical elongation of cell in plants is regulated by the auxin, a plant hormone which can promote or inhibit it. The redistribution of the auxin determines the direction of the response to an external stimulation (Girloy, 2008; van Overbeek, 1939). Hygroscoy and drying concern the ability of a tissue to absorb the water molecules from the surrounding environment or expel them leading to a change in volume of the cell membrane, which depends on the degree of moisture in the surrounding.

Movement in plants is affected by both endogenous and exogenous factors. An endogenous movement is a biological process, such as the circadian rhythms, which lead changes within the plant structure although external conditions remain constant. Exogenous movements are responses which are elicited by external elements such as the light (i.e., phototropism), the gravity (i.e., gravitropism) or the direction of the sun (i.e., heliotropism). In general, movements are classified in two main classes namely nastic and tropic movements which refer to the directionality of the response to both endogenous and exogenous cues. The tropic response is strongly related to the direction of external factors while in the nastic response the direction is influenced by the features of the tissue, and it is independent by the origin of the stimulus. In the following sections, both tropic and nastic movements will be described in detail as to provide an overall picture of "*The power of movement in plants*".

2.2.1. Tropic movements

Plants are sessile organisms which have evolved exceptional strategies to adapt to the challenges of their surroundings. For instance, plants must explore the environment

searching for nutrients and water, or they must respond to herbivory and pathogen attacks efficiently. To cope with these no easy tasks plants must have the ability to code and process external information, and to direct and control their growth toward an external stimulus as to enhance their chances to survive (Mancuso & Viola, 2013). Plants show a variety of tropic behaviors in response to different environmental elements (e.g., light, temperature, gravity, ...) that will be described in detail in the following sections.

Phototropism

Phototropism is defined as the tendency of plants to grow toward the light source, which can be distinguished between positively (i.e., bending of the organ of plants towards the light source) or negatively (i.e., bending of the organ of plants away from the light source) phototropic responses (van Overbeek, 1939). The plant's stems are of the first type, while the roots are of the second type. The tropic response is elicited by the blue light, and it is mediated by specific proteins of photoreceptor (i.e., phototropins), which allow the plants to discriminate between various wavelengths of light (Girloy, 2008).

Charles Darwin (1880) advanced that the light perception system is located within the shoot apex of a plant and drives and regulates the tropic growth for all organs of a plant. To test this, Darwin and his son Francis observed the behavior of the canary grass (*Phalaris canariensis* L.) in five different experimental conditions (Fig. 2.1.): (a) a plant exposed to a light source (Fig. 2.1. a); (b) a plant exposed to a light source, but the below part of the stem was covered by a tube (Fig. 2.1. b); (c) the shoot apex of a plant was removed (Fig. 2.1. c); (d) the shoot apex of a plant was covered by a clear cap (Fig. 2.1. d); (e) the shoot apex of a plant was covered by a cap preventing the access to light (Fig. 2.1. e).

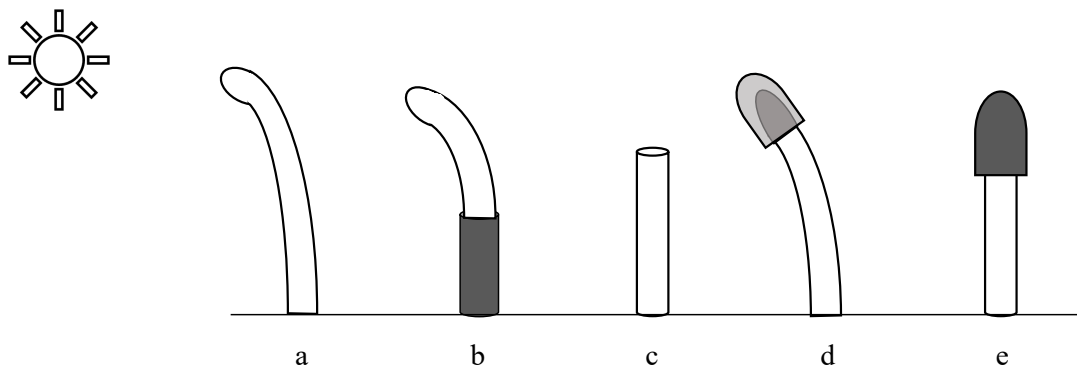


Fig. 2.1. Graphical representation of the experimental conditions used by the Darwins to test the sensitiveness of the shoot apex to the light source in the *P. canariensis* plant.

Darwin observed that all plants with the apex accessing light showed phototropism (i.e., ‘a’, ‘b’, and ‘d’ conditions), while when the apex was removed (i.e., ‘c’ condition) or covered (i.e., ‘e’ condition) the plant remained still. Results suggested that the sensory apparatus, that drives the tropic growth, may be located within the apex of a plant.

Heliotropism

The elongation and growth of the organs of the plant are regulated by the complex interactions between light, temperature, and circadian clock (Müller, von Korff & Davis, 2014; Nozue, Covington, Duek, Lorrain, Fankhauser et al., 2007). The coordination of both circadian clock and directional growth is the best strategy to capture a great amount of light. In general, this process is regulated by both the phototropism and heliotropism, or solar tracking. However, heliotropism is the more dynamic process, which allows the plants to follow the movement of the Sun throughout the day by means of their aerial part. The most famous example of heliotropic movements is the sunflower (*Helianthus annuus* L.). The shoot apices shift from east to west during the day and then reorient at night to

east in anticipation of the dawn (Atamian, Creux, Brown, Garner, Blackman & Harmer, 2016).

Gravitropism

Another environmental factor used by plants as a guide for growth is gravity (Knight, 1806). The gravity-directed growth process (i.e., gravitropism) ensures the proper positioning of the shoot and leaves for an efficient photosynthesis and directs the growth of the root system in the soil to achieve nutrients and water (Hashiguchi, Tasaka & Morita, 2013; Morita, 2010; Morita & Tasaka, 2004; Su, Gibbs, Jancewicz & Masson, 2017; Toyota & Gilroy, 2013). For instance, if we move a plant from the vertical to the horizontal plane, after some hours we will notice that the leaves will start to reorient their growth upward toward the light source. There are three different types of gravitropism: (i) negative, when the plant shoot grows against gravity; (ii) positive, when the root system penetrates the ground growing towards the direction of gravity; (iii) transversal, when the tree branches grow parallel to the direction of gravity (Knight, 1806). It has been demonstrated that the columella cells of the root cap play a fundamental role in perceiving the changes in the gravitational field. Then, the perceived information is transduced in physiological signals and transmitted to the whole plant's body promoting a different cellular elongation and curvature of the plant's organs (Ciesielski, 1872; Darwin & Darwin, 1880).

Hydrotropism

One of the most important functions of the root system is to gain nutrients. To satisfy this need, roots have evolved different strategies to sense water gradients and to use this information to modulate the root's growth toward the richest patches. This movement

toward the water source is named hydrotropism and a possible candidate underlying this tropic response might be the hormone auxin. Studies on the investigation of the mechanism underlying hydrotropic sensing led to contrasting results given by the continuous effects of gravity on the plant growth (Dietrich, 2018; Takahashi, Goto, Okada & Takahashi, 2002). To deal with this problem, mutant plants (e.g., *Arabidopsis thaliana* L. or *P. sativum* mutants), which are neither gravitropic nor phototropic, but do respond to a moisture gradient, are employed (Jaffe, Takahashi & Biro, 1985; Mizuno, Kobayashi, Fujii, Yamashita & Takahashi, 2002; Takahashi et al., 2002; Takahashi, Yamazaki, Kobayashi, Higashitani & Takahashi, 2003). For instance, a study on the root of mutant *P. sativum* “*Ageotropum*” showed that when the root cap was removed the hydrotropism response was affected in contrast to the elongation of the roots. These results demonstrated the role of the root cap for sensing and coding the moisture gradients (Dietrich, 2018; Hooker, 1915). In sum, testing mutant plants with defects in gravitropism, auxin or in the hydrotropism response represent a useful tool to differentiate between the hydrotropic response and gravitropism and to provide elucidation about the mechanism for the hydrotropism in plants. However, further studies are needed to better understand hydrotropism, the mechanism underlying it and how it contributes to water uptake and drought responses in plants.

Thigmotropism

Along with hydrotropism, there is the thigmotropism, namely “*the tendency to respond to mechanical contact by clinging and curving.....*” (Telewski, 2012). Thigmotropic responses are typically exhibited by climbing plants’ organs such as tendrils, roots, or the stem, which present a growth response toward the point of stimulation (Darwin, 1875). For instance, the thigmotropic response of the tendrils (i.e.,

modified leaves) or the stem is utilized by climbing plant to secure their grip toward a potential support and climb it to achieve a vertical height and enrich the maximum light exposure (Braam, 2005). Another example is provided by the root system whose apex is touch-sensitive and it allows the transmission of the signals provided by the touch stimulation to the proximal root regions leading to the change of growth direction. Many flowers have evolved touch-sensitive organs (e.g., petals and pistils) to prevent self-pollination. For instance, some flowers present spring-loaded mechanisms that trigger explosive responses to spread seeds far away from the “*mother*” (Simons, 1992). Morphological changes induced by touch stimulation implicate a huge number of inter and intracellular signaling components such as hormones and potential second messengers (e.g., intracellular calcium - Ca^{2+} ; Batiza, Schulz & Masson, 1996; Calaghan & White, 1999). However, the primary signal which induce and control the thigmotropic response has not yet been identified.

2.2.2. Nastic movements

Nastic movements are defined as movements in response to an environmental stimulus whose direction is independent with respect to the stimulus position. The nastic response can be provoked by external stimuli such as (i) the temperature (i.e., thermonasty); (ii) the light (i.e., photonasty) or (iii) the mechanical stimulation (i.e., seismonasty). The thermonasty occurs in plant's organs depending on variation of temperature. An example could be the early blooming of the flowers in an environment with high temperature. The photonasty depends on the variation of the light which induces the opening or the closure of the leaves during the day/night period. The seismonasty occurs from a mechanical stimulation and some of the most interesting nastic movements in plants concern the closing of the leaf of the *D. muscipula* plant which was defined by

Charles and Francis Darwin (1888) as the “*one of the most wonderful plant in the world*”.

The *D. muscipula* is a carnivorous plant presenting two leaves with needle-shaped tines on the leaf margins. When an insect bumps into the bi-lobed leaves and touches the small hairs located within the leaves several times, intercellular electrical signals are generated triggering the closure of the leaves and trapping the prey (Fig. 2.2.; Burdon-Sanderson, 1873; Jacobs, 1954; Jacobson, 1965; Simons, 1981).

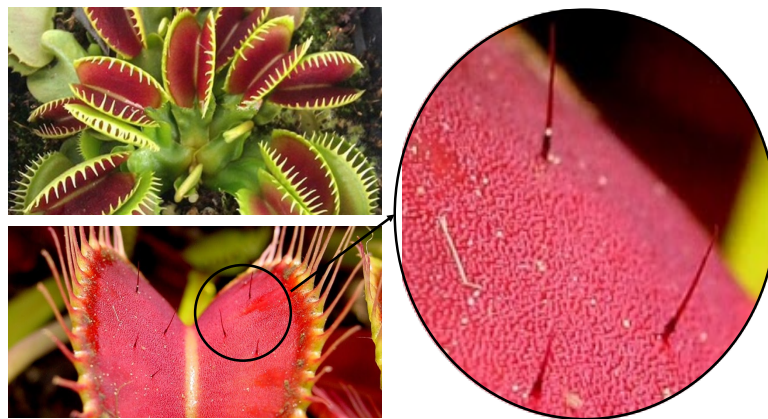


Fig. 2.2. Photograph showing the *D. muscipula* plant which can catch insects by closing its bi-lobed leaves once the prey touches the small hairs into the leaves several times.

Another interesting nastic movement is the rapid closing of the leaves of the *M. pudica* when they are touched by an external element considered by the plant as dangerous (Fig. 2.3.).



Fig. 2.3. Photograph showing the folding of the leaves of the *M. pudica* plant when they are touched by an external stimulus (e.g., a human finger) considered by the plant as dangerous.

The mechanical stimulation induces an electric signal in the touched leaf which spreads to the cells of pulvini, specialized motor organs located at the bases of the leaflets and petioles, leading a variation in the turgor pressure, and provoking the closing of the leaves (Malone, 1994; Simons, 1981). Interestingly the velocity of the folding of the leaves depends on the intensity of the mechanical stimulation. For instance, if the leaves are touched by a human finger the folding of the leaves will be faster compared to when they are touched by a little heat source.

2.3. On the universality of plants' movements: circumnutation

Darwin and Darwin (1880) identified a pattern of movement common and universal among all plants (i.e., circumnutation), during which plant's growing organs are described as circular or elliptical movements around their central axis (Fig. 2.4.).

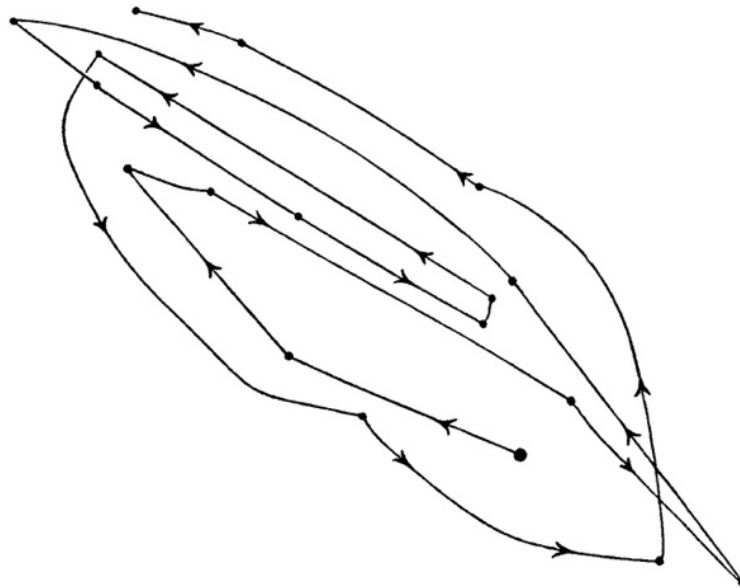


Fig. 2.4. Graphical representation of the trajectory of the circumnutation movement of the shoot of the *Brassica oleracea* L. along 10 hours and 45 minutes. (from Darwin & Darwin, 1880).

The Darwins observed that the properties of circumnutation (e.g., movement direction, amplitude, ...) may vary across age and species. Indeed, the plant's growth may be characterized by vigorous or by no oscillations, as circumnutation may be interrupted

for several hours. The direction of the circular movement could be clockwise or counterclockwise (Fig. 2.5.) and it could change during the same or different days.

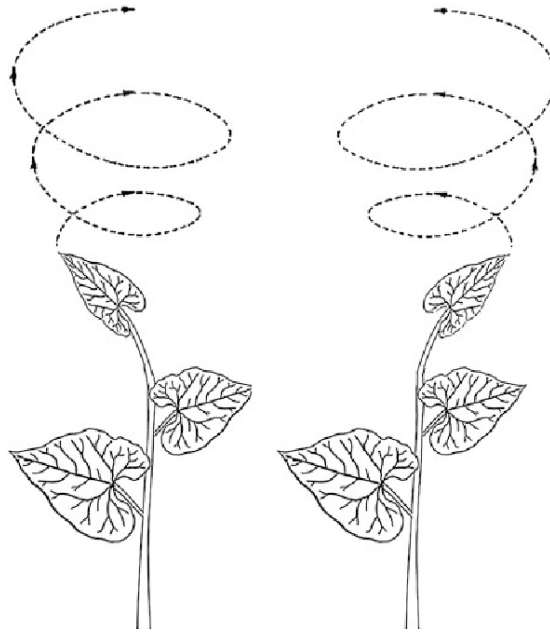


Fig. 2.5. Graphical representation of circular movement by the stem of a Bean plant (*Phaseolus vulgaris* L.) in clockwise (left side) and in counterclockwise (right side). (from Mugnai et al., 2015)

For example, the direction of the circumnutation in shoots of *A. thaliana* plant is usually in clockwise (Schuster & Engelmann, 1997), while shoots of *P. vulgaris* displays counterclockwise direction (Millet, Melin & Badot, 1988). Then, touch and geotropic stimulation may induce changing in direction of the rotation (Okada & Shimura, 1990; Stolarz, Dziubinska, Krupa, Buda, Trebacz & Zawadzki, 2003). The number of rotations around the central axis of the plant vary between different plant species. For examples, the plant of *B. oleracea* is used to perform four rotations in twelve hours, while the plant of *Opuntia basilaris* Engelm. & J.M. Bigelow performs one cycle in the same period. Furthermore, Darwin noticed differences also within the same plants during the growth phase. Indeed, in most seedling the circular movement was smaller and faster since the complete development of the first leaves. Also, the amplitude of the circumnutation varies between different plant species and it is not related with the length of the circumnutating plants' organs, but it depends on the presence of various external stimuli

(e.g., gravity; Brown, Chapman, Lewis & Venditti, 1990; Millet et al., 1996).

Furthermore, the period of circumnutation may last between several minutes to hours, and it depends on morphological features, environmental conditions (e.g., temperatures, light/dark period, ...) and elements (e.g., gravity, chemical elements, ...; Johnsson, Haupt & Feinleib, 1979).

Since Darwin's discoveries, the nature of the mechanism underlying circumnutation has been debated (Darwin & Darwin, 1880). To date, three main models have been advanced: endogenous origin, the result of earth gravity and both endogenous and exogenous processes (Kiss, 2006; Mugnai, Azzarello, Masi, Pandolfi, Mancuso & Shabala, 2007; Stolarz, 2009). In the first model, it has been stated that the driving and the regulator process of circumnutation movement is internal to the plant, which can modify this process for its advantage (Darwin & Darwin, 1880). Different hypotheses have been advanced to explain the endogenous nature of circumnutation: (i) a periodic variation in auxin fluxes from the tip (Arnal, 1953); (ii) changing in sensitiveness of elongating cell to auxin (Joerrens, 1959); (iii) each plant cell has an internal "*cellular nutational oscillator*" (Heathcote & Aston, 1970); (iv) the existence of an intrinsic oscillator given the great correlation founded between circumnutation movement and the rhythmical patterns of ion fluxen in corn's roots (*Zea mays* L.; Shabala 2003; Shabala & Newman 1997). In the second model, circumnutation movement is generated by the presence of an external stimulus as the gravity of the earth (Kiss, 2006; Kitazawa, Hatakeda, Kamada, Fujii, Miyazawa, Hoshino, et al., 2005). In line with this view, Israelsson and Johnsson (1967) advanced a gravity-dependent model for explaining the nature of circular nutation in plants. They considered circumnutation as gravity driven, in which oscillations are interpreted as continuous over-compensatory responses to the changing orientation of plants' organs with respect to the position of the Earth's gravity vector. In this view,

circumnutation is concerned as a special kind of tropic behavior. However, experiments conducted in microgravity environments demonstrated that gravitropism and circular nutation are independent processes. Indeed, the gravity appears not to be a fundamental element for the mechanism underlying circumnutation of the growing roots (Paul, Amalfitano & Ferl, 2012). In the third model, it has been suggested that circumnutation is induced by both endogenous and exogenous factors (Johnson, 1979; Johnsson, Jansen, Engelmann & Schuster, 1999; Johnsson, et al., 1979). The circular oscillations are internally generated by the plants, but they could be affected by the gravity (Britz & Galston, 1982; Orbovic & Poff, 1997). A possible relationship between gravitropism and circular nutation movement has been advanced by Yoshihara and Iino (2005; 2006). They demonstrated that the circular oscillations of the rice (*Oryza sativa* L.) coleoptiles were affected by a gravitropic response, even if gravitropism was not involved in the process of circumnutation.

But what is the role of circumnutation in the life of a plant? It has been advanced that circumnutation may be used by the plant to explore the environment as to find elements useful for ensuring survival, for instance a support, as in the case of climbing plants (*see next section*; Darwin, 1875; Gianoli, 2015; Stolarz, 2009). Otherwise, circular movements performed by the primary root (or radicle) might be used by the plant to penetrate through the ground during the elongation phase (Darwin & Darwin, 1880; Inoue, Arase, Hagiwara, Amnao, Hyashi & Ikeda, 1999; Minorsky, 2003; Schuster & Engelmann, 1997). The radicle is the first part that emerges from the seed during the germination phase, which immediately bends towards the soil to penetrate it (Fig. 2.6.).

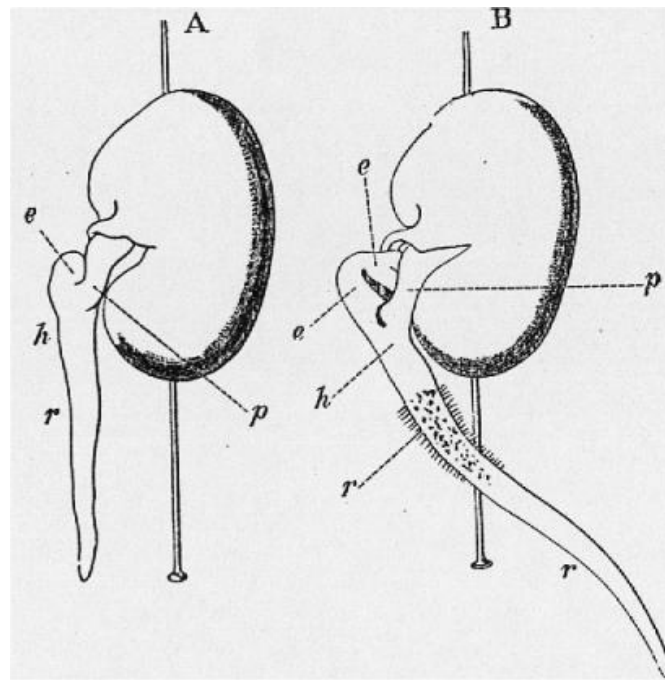


Fig. 2.6. Drawing made by Charles Darwin (1880) representing the germinating seed of *V. faba*. In panel A is showed a radicle growing perpendicularly downwards, while in panel B it is represented the curvature of the radicle after a period of 24-48 hours from the seed germination. Note: r = radicle; h= short hypocotyl; e = epicotyl; p = petiole of the cotyledon. (from Darwin & Darwin, 1880).

Subsequently, the radicle starts to circumnutate to explore the soil searching for a path less resistant and to deal with the heterogeneity of soil conditions (Gandar, 1983; Massa & Girloy, 2003a; Semchenko et al., 2008; Taylor, Lehner, McCaskey, Nirmal, Ozkan-Aydin, Murray-Cooper et al., 2021). Studies on *A. thaliana* (Mirza, 1987; Mullen, Turk, Johnson, Wolverson, Ishikawa, Simmons et al., 1998; Okada & Shimura, 1990; Simmons, Migliaccio, Masson, Caspar & Söll, 1995a; Simmons, Söll & Migliaccio, 1995b), *P. sativum* (Spurny, 1966) and *Z. mays* plants (Ney & Pile, 1981) demonstrated that roots make a symmetrical spiral helix to penetrate the soil, which is more prevalent on clockwise direction. Indeed, even if the movement direction of shoots has been reported as irregular, in the root's tip prevalent direction is on the right-side. Recently, it has been suggested that roots movements may be generated by the combination of three different processes, such as circumnutation, gravitropism and negative thigmotropism (Migliaccio & Piconese, 2001). However, results from different studies do not converge in a single

direction. Indeed, it has been demonstrated that the root movement depends only on the circumnutating movement in conditions without gravitropism and negative thigmotropism (Piconese, Tronelli, Pippia & Migliaccio, 2003).

Darwin was completely fascinated by the movement of the root tip, in his words: “*We believe that there is no structure in plants more wonderful, as far as its functions are concerned, than the tip of the radicle*”. Darwin discovered that the tip, when it is burnt, cut or it got in touch with external stimuli (e.g., the light, touch by an object, ...), sends to the aboveground part of the plant a signal, causing a bending in the opposite direction from the affected side. Furthermore, he believed that the tip of the radicle could determine and guide the movement of secondary roots (i.e., roots starting from the central radicle) into the ground, given its great environmental sensitiveness. By showing a remarkable power of action and sensitiveness, the tip of the radicle can perceive and respond to the presence of a variety of factors in the soil such as obstacles, resources distribution and/or competitors (Cahill & McNickle, 2011; Cahill et al. 2010; Clark, Whalley & Barraclough, 2003; Hodge, 2004; 2009; Kozlowski, 1999). Several studies have, indeed, reported that in the presence of a heterogeneous soil nutrient distributions, plants would choose to place more roots in the highest nutrient-rich patch (i.e., foraging precision strategy; for a review see Cahill et al., 2010). Furthermore, it has been demonstrated that plants are also able to sense the variability in soil resources, to evaluate the risk associated to a specific situation and to make decision to maximize their fitness in a similar manner observed in different animal species, including human beings (Caraco, Martindale & Whittam, 1980; Dener et al., 2016; McNamara & Houston, 1992). In sum, it seems that all plants’ movements are a form of modified circumnutation which is a fundamental process for the plants’ life, and it could be defined as a pure manifestation of plant behavior.

2.3.1. Modified circumnutation: the case of climbing plants

It was in the years 1862-1863 that Charles Darwin began his studies on the movement of climbing plants. Darwin became interested in this topic by reading the paper “*Note on the coiling of the tendrils on plants*” by Asa Grey (Darwin, 1875). Since then, the nature of climbers became one of Darwin’s greatest “*loves*” and a much-debated topic between him and the famous botanist Joseph Dalton Hooker, with whom Darwin had a constantly epistolary correspondence on climbers’ behavior (Fig. 2.7.).

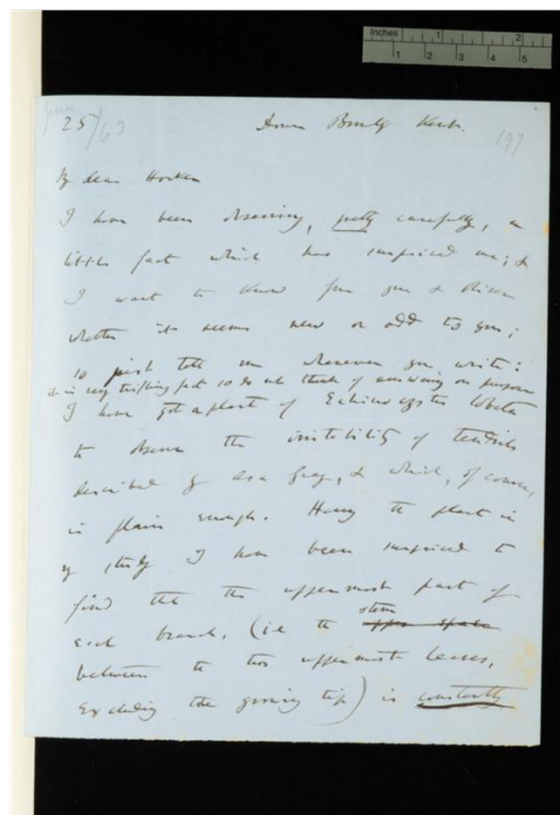


Fig. 2.7. Original letter by Charles Darwin to J.D. Hooker (date: June 25, 1863) in which Darwin described his first observation of the circular nutation of the tendrils in climbing plants. (from Cambridge University Digital Library, Cambridge, England).

Darwin was totally fascinated by the ability of climbing plants to evolve and adapt their behavior to an ever-changing environment to maximize their possibilities to survive. Indeed, climbing plants are not self-supporting, so they need to find a potential element into the environment to growth vertically as to reach the light.

Darwin (1875) identified four major classes of climbing plants, namely twining plants, which are only able to coil around a support by means of the stem, leaf- and tendrils-climbers, which use irritable organs to anchor themselves to a surface, and root- and hook-climbers, which adhere to the trunk of trees or to different surfaces by means of root hairs on the stem. Twining plants was the first subject treated in the book “*The movements and habits of climbing plants*” by Charles Darwin (1875). Darwin was impressed by the revolving movement of the climbers’ stem which bends to one side to another to find a potential support to climb and, once it has been detected, it starts to coil and grow spirally around the support (Darwin, 1875; Palm, 1827; Fig. 2.8.).

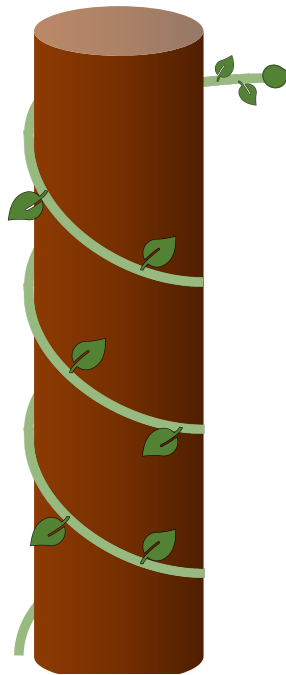


Fig. 2.8. Graphical representation of a spirally coiling stem in “*action*”.

But how does the coiling movement around a support occur? It has been advanced by von Mohl (1827) that the stem of twining plants may have a kind of irritability, which causes the natural tendency of twining plants to coil and grow spirally around a support after touching it (Darwin, 1875; von Mohl, 1827). However, Charles Darwin, Ludwig H. Palm and Henri Dutrochet were not on the same page (Darwin, 1875). These authors

claimed that the coiling movement around a support must be considered as the pursuit of the circumnutation movement of the shoot. Indeed, once the tip of the shoot touches the support, the plant continues to circumnutate and to grow vertically on the support. Moreover, each internode of the growing stem stops to move when it becomes old. The union of the revolving and the vertical growing movement produces the shaping of the stem into a spire around the support (Fig. 2.8.). Therewith, Darwin (1875) noticed that the first internode tends to perform a close spire on the support securing the plant attachment during climbing, while the following spires are used to be more open (Putz & Holbrook 1992; Silk & Holbrook, 2005). To sum up, the twining movement around a support seems to be the result of the circumnutation movement of the stem. To clarify this concept, Darwin (1875) use this analogy to describe the coiling movement of the stem: *“If a man swings a rope round his head, and the end hits a stick, it will coil round the stick according to the direction of the swinging movement; so it is with a twining plant, a line of growth travelling round the free part of the shoot causing it to bend towards the opposite side, and this replaces the momentum of the free end of the rope”*.

The second class of climbing plants described by Charles Darwin was the type of plants that use irritable organs to climb environmental stimuli, namely leaf-climber and tendrils-bearers. The first subclass collects all those plants, that present sensitive petioles of the leaves as *Clematis* plants (*Clematis glandulosa*; *Clematis viticella* var. *venosa*; Fig. 2.9.) and the Jasmine nightshade plant (*Solanum jasminoides* Paxton). Darwin believed that the purpose of the circular movement was to bring the leaves into contact with potential supports for ensuring the adhesion of them to their surface.

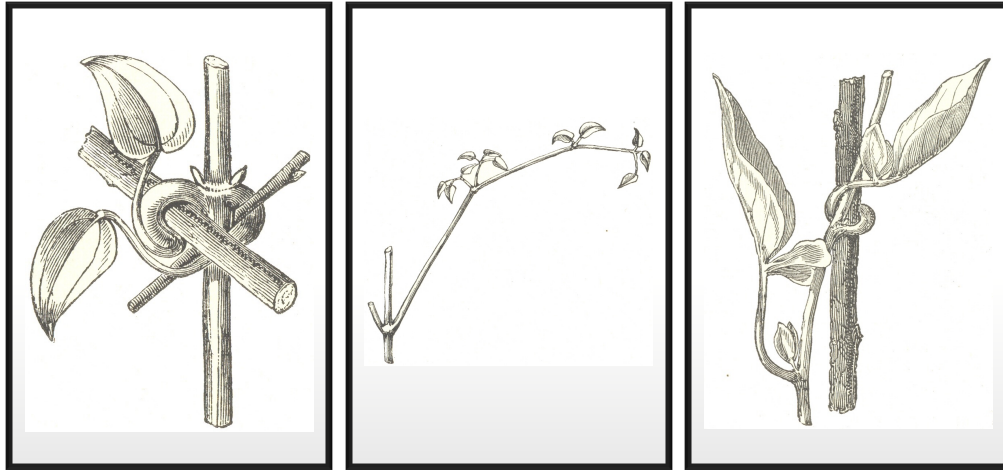


Fig. 2.9. Drawing made by Charles Darwin, in which three types of leaf-climber plants, namely *C. glandulosa* (left-side panel) and *C. viticella* (central panel) and *S. jasminoides* (right-side panel) are represented. (from Darwin, 1875).

In the second subclass, Darwin described those plants that climb a support by means of the tendrils, that is “*filamentary organs, sensitive to contact and used exclusively for climbing*” (Fig. 2.10.; Darwin, 1875). Darwin described the tendrils as a clear example of how the natural selection operates. Indeed, the tendrils are modified leaves which are used by the plant to climb different surface and adapt its morphology to survive and cope in different environments (Darwin, 1875; Palm, 1827; von Mohl, 1827).

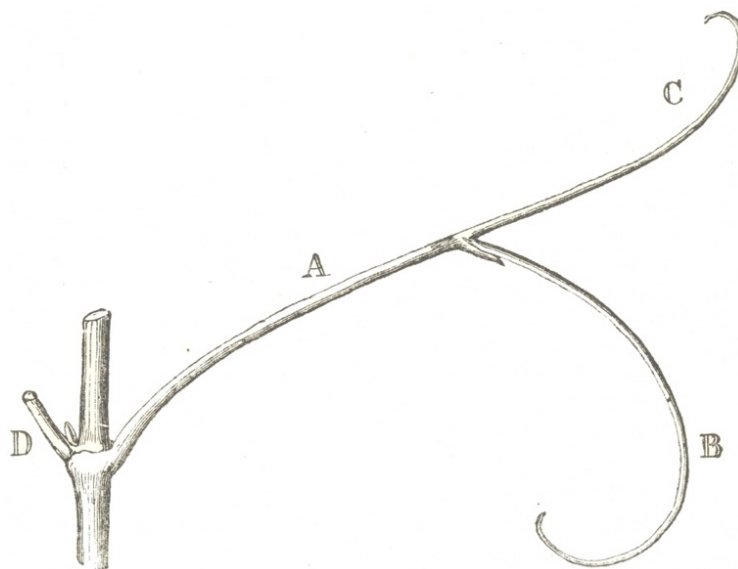


Fig. 2.10. Darwin’s drawing in which the tendrils (B, C) of a *Vitis* plant (*Vitis vinifera* L.) are represented. Note: A= the peduncle from which the tendrils develop; D = the stem of the *Vitis* plant. (from Darwin, 1875).

Darwin noticed that the tendrils have their own revolving movement, and they are sensitive to touch which induces a curvature in the direction of the touched side. Some tendrils are sensitive on both ventral and dorsal surfaces, while other only on the ventral surface. In general, tendrils present three types of movement: (i) circumnutation, which is defined as an internal movement of the growing tendrils which is used to increase the possibilities to get into touch with a potential support; (ii) contact coiling, which refers to the curvature of the tendrils in response to a mechanical stimulation. This movement is affected by the force of impact and the type of support's surface; (iii) free coiling, which is defined as the helical coils developed along the tendril's axis (Darwin, 1875; Jaffe & Galston, 1968a, b; MacDougal, 1893). Furthermore, it has been demonstrated that the tendrils are selective in their response to a mechanical stimulation, which could be affected by the presence of different environmental conditions (e.g., temperature, the light, ...; Jaffe, 1980; Jaffe & Galston, 1967a, b, c; 1968a, b). For instance, it has been demonstrated that the water at natural or low temperature does not induce the shaping into curvature of the tendrils, while hot water (i.e., 40° C) it does. Host and support features such as roughness may also affect the way of climbing in plants (Putz, 1984; Putz & Chai, 1987). For instance, the *P. vulgaris* increases the stem's axial twisting with support roughness (Darwin, 1875). Darwin (1875) also noticed that the tendrils are capable of self-/non-self-discrimination, which leads tendrils not to react with tendrils of the same plant. In general, climbing plants tend to grow rapidly in both vertical and horizontal directions and their tendrils can come in touch with their own and others leaves and stems. Climbing plants tend to compete with neighbor plants by blocking the access to the light (Gentry, Putz & Mooney, 1991; Paul & Yavitt, 2011). In this view, the ability of tendrils to discriminate between self and non-self seems to be necessary to avoid self-coiling providing an advantage over neighboring plants in competition for light and space. It has

been demonstrated that the perennial vine *Cayratia japonica* (Thunb.) has a self-discrimination ability by means of its tendrils. Fukano and Yamawo (2015) compared the coiling responses in *C. japonica* plant toward neighbors in different experimental conditions, namely (i) physiologically connected self plants (i.e., plants could be connected via stems, rhizomes, ...), (ii) physiologically separated self plants and (iii) non-self plants. Results showed that *C. japonica* plants were more likely to coil with their tendrils around neighboring plants not physically connected compared to the connected ones. These results suggested that the ability of self-discrimination for plant competition does not occur only underground, but also aboveground (Fukano & Yamawo, 2015).

Darwin (1875) described other two classes of climbing plants namely the hook- and root-climbers. Hook-climbers are plants with long and weak shoots, by which plants can climb over other plants. While root-climbers are climbing plants, which attach their structure to walls and trees by secreting a kind of glue from their root hairs, which are developed from the stem (e.g., ivy plant - *Hedera helix* L.; Fig. 2.11.).



Fig. 2.11. Photograph showing the root-climber *H. helix* plant, which can climb different surfaces such as a house wall.

Hook- and root-climbers represent an example of how nature is able to evolve new strategies as to cope and to adapt to different environments (e.g., desert) in which trellis availability is limited (Molina-Freaner & Tinoco-Ojanguren, 1997). It has been discovered that plants' needing to find a potential support to climb leads changes in fitness, growth form and morphology as well (Gianoli, 2001; 2003; 2015). Furthermore, trellis availability and host trees properties may affect the distribution of climber diversity into the environment (Carrasco-Urrà & Gianoli, 2009; Darwin, 1875; Garbin, Carrijo, Sansevero, Sánchez-Tapia & Scarano, 2012; Hegarty, 1991; Putz, 1984). For example, it has been demonstrated that the roughness and the diameter of the cortex of host trees may affect the probability of infestation by climbers (Carsten et al., 2002; Darwin, 1875; Gianoli, 2015; Putz, 1984; Putz & Chai, 1987; Silk & Holbrook, 2005).

In conclusion, climbing plants are a perfect example of how plants are not only able to display various types of behavior in response to different environments but also to modify them in order to achieve and satisfy their “*wants*”.

2.4. Methods and techniques for the study of movement in plants

Charles Darwin (1880) was the first who described in detail the movement of plants by tracking the changes of position through time and space of the shoot apex in a variety of plants. This was the very first example of kinematical study on plant's nutation. Since then, several studies on different kinematical aspect (e.g., oscillatory shapes and directions, amplitude, period, ...) of nutation's movements in different plant's organs (e.g., the root system, shoot, apex, ...) have been conducted by means of time-lapse images and video processing to extract nutation-related features (Brown, 1993; Calvo, Raja & Lee, 2017; Migliaccio et al., 2013; Millet et al., 1988; Mugnai et al., 2015; Raja et al., 2020; Stolarz, 2009). The main feature of this methodology is to measure how each tracked point

of interest moves. The extraction and the analysis of the nutation-related features of different plant's organs lead to investigate the underlying mechanisms of such movements such as internal oscillators, gravitation-driven mechanisms, or the combination of these two mechanisms (Brown, 1993; Johnsson & Israelsson, 1968; Stolarz, 2009). In this view, the development of novel techniques aimed to analyze the movement of both the above- and belowground plants organs would be useful for the studying and the understanding of the variety of behaviors showed by plants. For instance, the kinematical analysis of movement of the belowground organs such as the root system provides the study of spatiotemporal patterns of the growth and curvature of the roots. A variety of tools have been developed to this task such as the KineRoot (i.e., Basu, Pal, Lynch & Brown, 2007), the SimRoot (i.e., Lynch, Nielsen, Davis & Jablonsky, 1996), the RootTrace (i.e., French, Bennett, Howells, Patel & Pridmore, 2008; French, Ubeda-Tomás, Holman, Bennett & Pridmore, 2009) and the analyzer for root tip tracks (i.e., ARTT; Russino, Ascrizzi, Popova, Tonazzini & Mancuso, 2013). RootTrace allows for the study of the gravitropic response of the primary root by tracking its growth in the downward direction. Another example is provided by ARTT which allows for the extraction of the kinematical features of the movement of the root tips from the sampled tip positions by means of the acquisition of a set of images during plant growth. Then, the software provides a graphical output of tracks and a textual output of kinematical features which can be used for statistical analyses. This software allows for the study of the plant-root behavior and kinematics among and across different plant species such as *Z. mays* and *O. sativa* (Russino et al., 2013). Recently, novel studies on the three-dimensional (3D) reconstruction of plant-root architecture have proposed new ways for quantify the growth process (e.g., Clark, MacCurdy, Jung, Shaff, McCouch, Aneshansley & Kochian, 2011; Fang, Yan & Liao, 2009; Mairhofer, Zappala, Tracy, Sturrock, Bennett, Mooney &

Pridmore, 2012). However, these studies are mainly focused upon the architectural and phenotypical analyses of the root system, thus omitting the kinematical aspect in toto.

Concerning the analysis of the movement of the aboveground plant's organs such as the apex and the stem, Stolarz and colleagues (2014) implemented a software for plant circumnutation 2D analysis (i.e., Circumnutation Tracker) which allows for the manual extraction of the kinematical parameters of the circumnutation movement by means of the analysis of time-lapse videos of the plant from a top view (Stolarz, Zuk, Król & Dziubinska, 2014). However, the Circumnutation Tracker software presents some limits such as (i) the manual extraction of the point's coordinates by the users could be error-prone and it represents a very heavy time-consuming process; (ii) the top view positioning of the cameras determines constraints on camera position; (iii) a 2D trajectory analysis precludes the recording of movements in other directions. Along these lines, some studies tried to improve the above constraints by adding side view contextual acquisition (i.e., Hatakeda, Kamada, Goto, Fukaki, Tasaka, Suge & Takahashi, 2003; Kosuge, Iida, Katou & Mimura, 2013; Schuster & Engelmann, 1997). They considered the automatization of the circumnutation analysis process (Stolarz et al., 2014) or implemented a possible approach for the reconstruction of the plant movement in three-dimensions (3D; Johnson, Solheim & Iversen, 2009). Indeed, the development of a software which consider the geometrical and local effect in the 3D space of the dynamic growth of all plant's organs as well as allowing for the extraction of the 3D kinematical features of plant's movement would be useful to better understanding the variety of plant's behavior and provide a full reconstruction of them (Bastien & Meroz, 2016; Gallentine, Wooten, Thielen, Walker, Speck & Niklas, 2020; Porat, Tedone, Palladino, Marcati & Meroz, 2020).

Raja and colleagues (2021) have developed a one-dimensional (1D) software, which is based on nonlinear methods for the analysis of the dynamics of plant nutation.

This approach, which is mainly based on the time dependencies characterizing the circumnutation pattern, may provide further information aside from the kinematical ones for the description and analysis of plant nutation. For instance, this methodology provides measurements about the biological and behavioral dynamics such as harmonicity, predictability, and complexity (e.g., Costa, Goldberger & Peng, 2005; Mottet & Bootsma, 1999)

More recently, a software for the analysis of plant movement allowing for the harvest and the extraction of circumnutation parameters in 3D has been implemented (Simonetti, Bulgheroni, Guerra, Peressotti, Peressotti, Baccinelli, et al., 2021). This software permits the study of the movement and behavior in a variety of plants by using principles similar to those used to study the kinematical features of movement in different animal species (e.g., Castiello, 2005; Sartori, Camperio-Ciani, Bulgheroni & Castiello, 2013; 2014). A stereovision system permits the acquisition of the photographs of plant's movement by means of a couple of fixed calibrated infrared cameras and the creating of the time-lapse videos of such movements. Then, a semi-automatic tracking process allows for the analysis of plant's movements and the reconstruct the 3D trajectory of the point of interest of the plant over the entire acquisition. Furthermore, the software analyzes the trajectory of the landmarks of the plant and compute the descriptive features of the 3D movement of the plant. This system has been developed in our laboratory and it has been utilized for the studies described in the present thesis to investigate the pattern of approach-to-grasp movement in *P. sativum* (Chapters 4, 5, 6, 7 and 8). A technical description is provided in Appendix.

PART II



THE EXPERIMENTS

CHAPTER 3



GENERAL METHODS

In the present chapter I will describe the methods and the procedures that are common to all the experiments included in my thesis. Exceptions will be reported within the “*Materials and methods*” section for each specific experiment.

3.1. Sample Description

P. sativum plants were chosen as plants’ model. They are annual climbing plants with tendrils, that are modified leaves used by plants to approach and grasp a suitable support (from now on “*stimulus*”) in the environment (Fig. 3.1.). Healthy-looking *P. sativum* seeds were selected, potted, and kept at the conditions outlined below. For each experiment plants were randomly assigned to the experimental conditions.

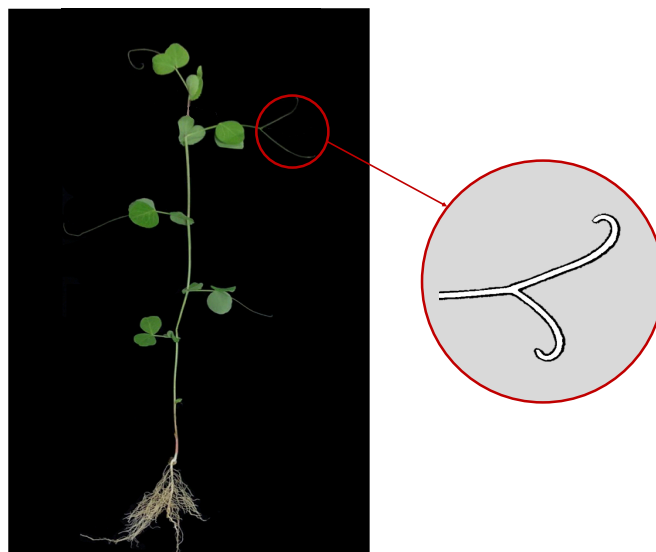


Fig. 3.1. A photograph representing a *P. sativum* plants. The call out is a graphical representation of the tendrils.

3.2. Experimental Stimuli

The stimuli were a wooden pole of a different thickness and shape positioned at 12 cm in front of the first unifoliate leaf for each plant.

3.3. Germination and growth Conditions

Cylindrical pots (diameter 20 cm; height 20 cm) were filled with silica sand (type 16SS, dimension 0.8/1.2 mm, weight 1.4). At the beginning of each experiment, pots were watered and fertilized using a half-strength solution culture (Murashige and Skoog Basal Salt Micronutrient Solution; 10x, liquid, plant cell culture tested; SIGMA Life Science). Pots were watered with tap water as needed three times a week. One seed per pot was placed at a distance of 6 cm from pot border and sowed at a depth of 2.5 cm. Individual pots were then enclosed in growth chambers (Cultibox SG combi 80x80x160 cm) for germination and growth in controlled environmental conditions (Fig. 3.2.).

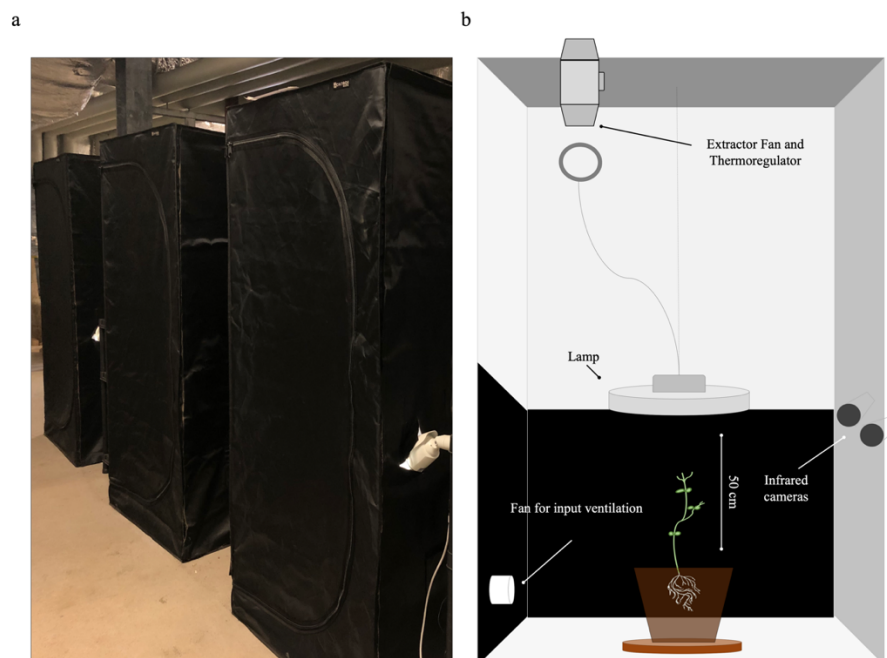


Fig. 3.2. (a) A photograph representing the external view of the growth chambers. (b) Schematic representation the experimental set up including: a couple of fans for the input and the extraction of the ventilation which allow a constantly air circulation in the growth chamber and the maintaining of a constant temperature; a cool white led lamp which was positioned above the pot at a distance of 50 cm; two infrared cameras for the videorecording of the plant's movement during the day and night period.

Chamber air temperature was set at 26 °C by means of an extractor fan equipped with a thermo-regulator (TT125; 125 mm-diameter; max 280 MC/H vents) and an input-ventilation fan (Blauberg Tubo 100 - 102m³/h). The combination of the two fans allowed for a steady air circulation into the growth chamber with an air mean residence time of 60 seconds. The disposition of the fan was such that air circulation did not affect the natural plants' movements. Plants were grown with an 11.25- hour photoperiod (i.e., 5.45 am to 5 pm) under a cool white led lamp (V-TAC innovative LED lighting, VT-911-100W, Des Moines, IA, USA or 100W Samsung UFO 145lm/W - LIFUD) that was centered at 50 cm above each seedling. Photosynthetic Photon Flux Density at 50 cm under the lamp in correspondence of the seedling was 350 $\mu\text{molPh m}^{-2} \text{ s}^{-1}$ (quantum sensor LI-190R, Lincoln, Nebraska USA). Reflective Mylar® film of chamber walls allowed for better uniformity in light distribution. Experimental treatments were applied to single plants.

3.4. Data recording and processing

For each growth chamber, a pair of RGB-infrared cameras (i.e., IP 2.1 Mpx outdoor varifocal IR 1080P) were placed 110 cm off the ground, spaced at a distance of 45 cm to record stereo images of the plant. The cameras were connected through Ethernet cables to a 10-port wireless router (i.e., D-link Dsr-250n) connected via Wi-Fi to a PC on which the frames acquisition and the saving process was controlled by means of CamRecorder software (Ab.Acus s.r.l., Milan, Italy). To maximize the contrast between *P. sativum* anatomical landmarks (e.g., tendrils) and the background for the sake of recording, black felt velvet was fixed on some sectors of the growth boxes walls and the wooden stimuli were darkened with charcoal. The intrinsic, extrinsic and the lens distortion parameters of each camera were estimated using Matlab Camera Calibrator app. The images dataset used for the single-camera parameters extraction process was created

by taking 20 pictures of a chessboard (squares' side 18mm, 10 columns, 7 rows) from multiple angles and distances, in natural non-direct light conditions. For stereo calibration, the same chessboard used for the single camera calibration process was placed in the middle of the growth chamber. Then, a picture was taken by the two cameras to extract the stereo calibration parameters. In the experimental protocol, a frame was acquired every 3 minutes (frequency 0.0056 Hz) synchronously from each camera. An ad hoc software (Ab.Acus s.r.l., Milan, Italy; see Appendix) developed in Matlab was used to position the markers, track their position frame-by-frame on the images acquired by the two cameras associated with each plant, and to reconstruct the 3D trajectory of each marker. Markers were inserted post-hoc on the anatomical landmarks of interest, namely the apex, the node below the tendrils, and the tips of the tendrils (Figure 3.3.). Markers were also positioned upon the stimulus (i.e., on both the lowest and the highest point of the stimulus), the bottom of the stem, the second leaf and the internode as reference points.

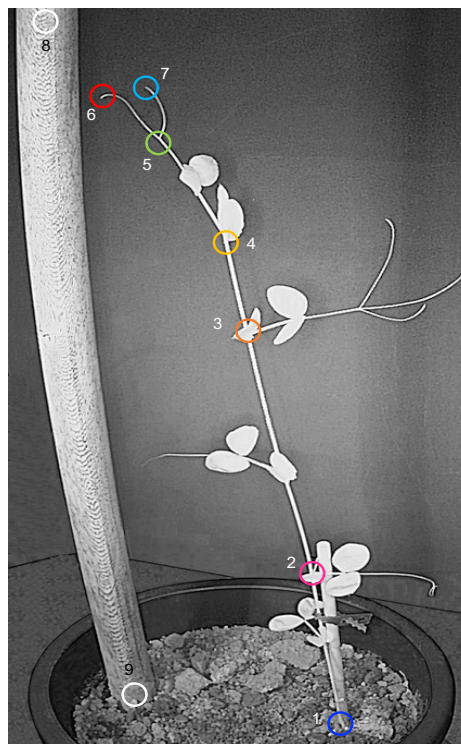


Fig. 3.3. A photograph representing the positioning of the markers on the considered landmarks of the plant, which were tracked in time and space for subsequent 3D kinematical analysis. Markers were inserted on: (1) the origin point of the plant; (2) the second leaf; (3) the internode; (4) the apex; (5) the node below the tendrils; (6,7) the tips of the tendrils and (8,9) the stimulus.

Tracking procedures were performed at first automatically throughout the time course of the movement sequence using the Kanade-Lucas-Tomasi (KLT) algorithm on the frames acquired by each camera, after distortion removal. The tracking was manually verified by the experimenter, who checked the position of the markers frame-by-frame. The 3D trajectory of each tracked marker was computed by triangulating the 2D trajectories obtained from the two cameras.

3.5. Dependent measures

Tendrils developing from different nodes were considered for the data analysis. For those cases in which the plant grasped the stimulus, the coiled leaf was analyzed. Whereas when no grasping occurred, the last node before the falling of the plant (Chapter 4) or the first node – starting from the bottom of the plant - characterized by two or three tendrils was examined (Chapter 7). The initial frame was defined as the frame at which the tendrils of the considered leaf were visible from the apex. The end of the plant movement was defined as the moment at which the tendrils of the leaf came in touch with the stimulus or the frame in which the tendrils fell down or remained still apart.

On the basis of pilot studies, the dependent measures specifically tailored to test the experimental hypotheses were:

1. The spatial trajectories of the considered point of interest of the plant. This measure was chosen because it provides a way for quantifying circumnutation in both qualitative and quantitative terms.
2. The average tendrils velocity (mm/min): the average velocity of the tips of the tendrils during circumnutation. This measure was chosen because it provides quantitative information about the average speed of the approaching tendrils movement with which the clasping of the stimulus must be completed.

3. The maximum tendrils velocity (mm/min): the maximum peak of velocity of the tendrils during circumnutation. This measure was chosen because it provides quantitative information about the peak of maximum speed of the tendrils during the approach phase toward a stimulus.
4. The time of maximum tendrils velocity (%): the time at which the tendrils reached the maximum velocity. This measure was chosen because it provides quantitative information in relative terms about the time spent during the acceleration phase.
5. The maximum tendrils aperture (mm): the maximum distance reached between the tip of the tendrils during the approach phase. This measure was chosen because it provides quantitative information about the anticipated size of graspable stimulus during the approaching movement of the tendrils.
6. The time of maximum tendrils aperture (%): the time at which the tendrils reached the maximum distance. This measure was chosen because it provides quantitative information in relative terms about the time at which the tendrils start to close upon the stimulus.
7. The movement time (min): the interval between the onset of the movement of the organ of the plant (e.g., wrist, tendrils, ...) and the end of it. That is, when the plant encountered the stimulus or fell down.

3.6. Data analysis

The median value of each considered dependent measures across condition were analyzed by means of the Wilcoxon rank-sum test (one-tailed). In addition to W -statistic and the p -value, we report the effect's size calculated as $r = z/\sqrt{N}$, in which z is the z-score and N is the total number of observations (Rosenthal, 1991). Statistical analyses were run using the computing environment R (R Core Team, 2014), and the function *wilcox.test*.

CHAPTER 4



THE APPROACH-TO-GRASP MOVEMENT: A KINEMATICAL STUDY

4.1. Introduction

As outlined above Charles Darwin (1875; 1880) was the first who described in detail the searching and approaching behavior of climbing plants towards a potential support. He stated that climbing plants use their circumnutation movement to explore the environment searching for a potential support and, once it is detected, plants adapt their approaching movement toward the support according to its structural properties. In other words, Charles Darwin (1875; 1880) assumed that climbing plants might be able to perceive an element in the surrounding and to modulate their behavior in a purposeful manner to achieve their goals. Since Darwin's observation, several studies have investigated the searching and attachment behavior in climbing plants at different level such as biomechanical (Bauer, Klein, Gorb, Speck, Voigt & Gallenmüller, 2011; Putz & Holbrook, 1992), cellular (Millet et al., 1988; Weiler, Albrecht, Groth, Xia, Luxem, Liß, et al., 1993), and physiological (Jaffe & Galston, 1968a, b; Stolarz, 2009). Other research on the approaching and grasping behavior in climbing and parasitic plants reports that they can locate both supports or host plants in the environment and to select among them the most suitable one depending on their needs (Carsten et al. 2002; Gianoli, 2015; Tronchet, 1945; 1946; 1977). For instance, it has been demonstrated that the dodder plant *Cuscuta pentagonata* Engelm., which need to attach itself on a host plant to gain nutrient as to increase its fitness, can both “*makes choices*” between different host species and modulate

its strategic coiling behavior around the stem of the neighboring plant based on the host (Kelly, 1990; Parise et al., 2021; von Mohl, 1827).

Despite such evidence, however, how plants can sense the presence of a support in the environment as to activate a suitable attachment behavior has yet to be uncovered. The question is if the plant actually can perceive different types of supports and if the movement changes, which aspects of the movement changes. The main aim of the present study was to fill this gap by investigating whether climbing plants can perceive an element in the surrounding and to plan and execute a movement according to the properties of such element (e.g., thickness).

To test this, the movement of *P. sativum* plant was assessed by means of 3D kinematical analysis in three experimental conditions: (i) in an environment lacking a stimulus (i.e., Condition 1), (ii) in an environment with a 3D stimulus that could vary in thickness (i.e., Condition 2), or in an environment with an ungraspable 2D picture of a stimulus (i.e., Condition 3).

4.2. Material and methods

The methods are identical to those described in Chapter 3 except for the exceptions that follows.

4.2.1. Sample and growth conditions

Twenty-five healthy-looking *P. sativum* seeds were selected (Table 4.1.), potted, and grown as specified within the “*Sample Description*” section of Chapter 3.

Table 4.1. Sample description

Condition 1		
Stimulus	3D stimulus	No stimulus
N°	5	5
Distance	12 cm	-
Germination period	5 d (\pm 0.8; Range 4 -6)	5 d (\pm 0.6; Range 5 – 7)
Age	15.6 d (\pm 2.4; Range 10 – 20)	21 d (\pm 4; Range 16 – 25)
Condition 2		
Stimulus	3D Thin	3D Thick
N°	5	5
Distance	12 cm	12 cm
Germination period	5 d (\pm 0.8; Range 4 -6)	6 d (\pm 1; Range 5 – 7)
Age	15.6 d (\pm 2.4; Range 10 – 20)	13 d (\pm 1; Range 12 – 15)
Condition 3		
Stimulus	2D Thin	2D Thick
N°	5	5
Distance	12 cm	12 cm
Germination period	6 d (\pm 1; Range 5 – 8)	7 d (\pm 0.8; Range 6 – 8)
Age	15 d (\pm 2.6; Range 11 – 19)	21 d (\pm 3.2; Range 14 – 22)

Note. Germination period and age, which are expressed in days, refer to the median, while median absolute deviation is noted in parentheses.

4.2.2. Experimental conditions

Condition 1

P. sativum plants were tested in an environment lacking a stimulus (Fig. 4.1. a) or with a stimulus, a wooden pole of 60 cm height and of 1.2 cm in diameter (Fig. 4.1. b).

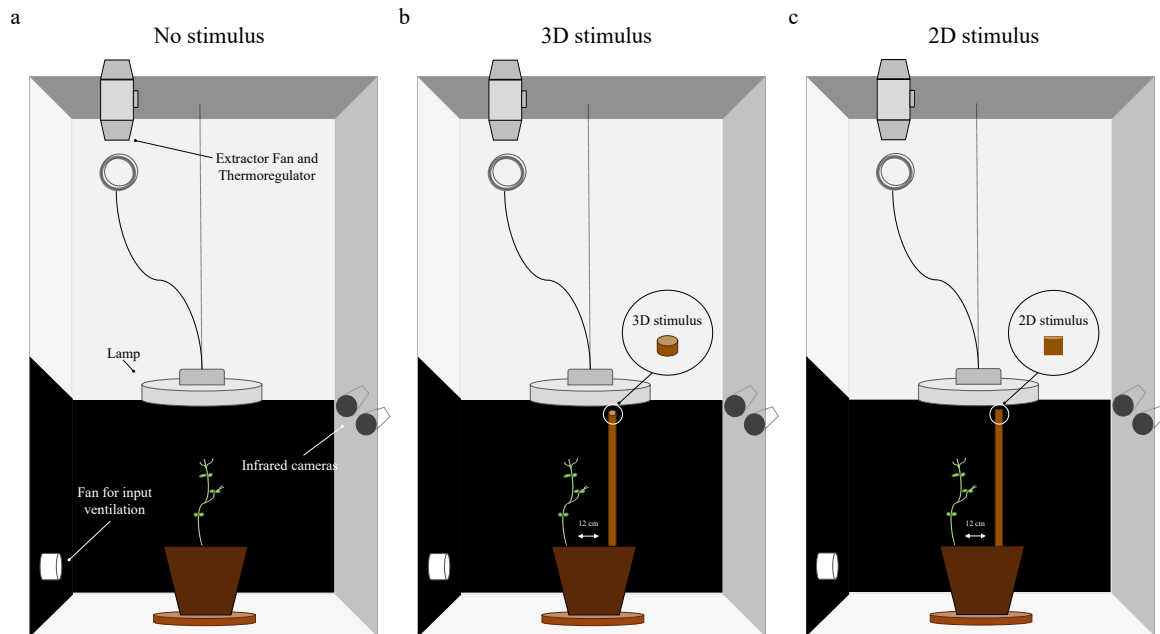


Fig. 4.1. Graphical representation of the experimental set up for the 3 experimental conditions (a) Condition 1 in which plant movement was assessed in an environment lacking a stimulus or toward a 3D stimulus; (b) Condition 2, in which plant movement was tested toward a Thin (i.e., 1.2 cm in diameter) or Thick (i.e., 3 cm in diameter) stimulus; (c) Condition 3 in which the ungraspable picture of the stimuli used in the Condition 2 was attached to one of the walls of the growth chamber.

Condition 2

P. sativum plants were tested with a stimulus which was a wooden pole of 60 cm height of either 3 cm or 1.2 cm in diameter (Fig. 4.1. b).

Condition 3

P. sativum plants were tested with 2D photographs of the 3D stimuli used for Condition 2 (Fig. 4.1. c).

4.3. Results

4.3.1. Qualitative results

The qualitative analysis of the three-dimensional spatial trajectories for the considered landmarks (Fig. 4.2. a, b and c) showed that the apex, the node below the tendrils and the tendrils presented a circular pattern of movement in all three conditions. In particular, the apex presented a less pronounced circumnutation movement compared to both the node below the tendrils and the tips of the tendrils. This pattern of movement was present since the early development of the tendrils. For all the three conditions, the plants started to circumnutate to explore the environment searching for a potential stimulus, but when the stimulus was not present (i.e., Condition 1; Fig. 4.2. a) or it was ungraspable (i.e., Condition 3; Fig. 4.2. c) they stopped moving and fell to the ground. Otherwise, when the plants perceived the presence of the stimulus (i.e., Condition 2; Fig. 4.2. b) they rapidly changed the direction of their circular movement toward the stimulus as to approach and grasp it. The search patterns exhibited by the *P. sativum* plants seemed systematic rather than random and might be equated to the systematic spiral search pattern that has been described for animals such as ants (Pfeffer, Bolek, Wolf & Wittlinger, 2015).

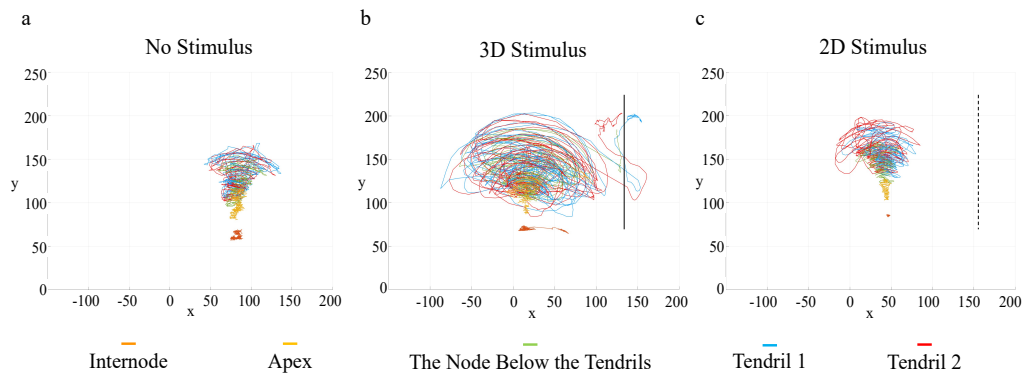


Fig. 4.2. The representative spatial trajectories of the considered point of interest of the plant: internode (orange), apex (yellow), the node below the tendrils (green), the tendrils (red and blue) for the Condition 1 (a), Condition 2 (b) and Condition 3 (c). The vertical lines represent the 3D (solid line) and the 2D (dashed line) stimulus. All plant's organs show a circular pattern of movement during their growth phase, which is more pronounced for the apex, the node below the tendrils and the tendrils. When the stimulus was no present (i.e., Condition 1) or was ungraspable (i.e., the 2D stimulus; Condition 3) the circumnutation movement of each plants' organs was directed toward the light source (a, c). While, when the stimulus was presented in the environment and it was detected by the plant, the tendrils veered towards the stimulus and stopped at the time grasping occurred (b). Axis x = sagittal axis in mm; axis y = vertical axis in mm.

4.3.2. Kinematical results

Condition 1

Results showed that the kinematics of the approach-to-grasp movement in *P. sativum* varies depending on the presence or the absence of the stimulus in the environment (Table 4.2.). More precisely, the average and the maximum tendrils velocity was higher for the 3D stimulus condition compared to the no stimulus condition (Table 4.2.). The time of maximum tendrils velocity and aperture occurred earlier for the no stimulus condition compared to the stimulus condition (Table 4.2.). No significant result was found for the maximum tendrils aperture when comparing the no stimulus and stimulus condition (Table 4.2.).

Table 4.2.

Kinematical and statistical values

Condition 1	Median		<i>W</i>	<i>p</i>	<i>r</i>
	3D Stimulus	No Stimulus			
Average tendrils velocity (mm/min)	1.90	0.88	28	.001	.57
Maximum tendrils velocity (mm/min)	12.13	5.24	43	.017	.43
Time of maximum tendrils velocity (%)	83.39	30.40	25	.001	.58
Maximum tendrils aperture (mm)	45.63	52.12	68	.268	.20
Time of maximum tendrils aperture (%)	88.50	32.76	7	.001	.62

Condition 2	Median		<i>W</i>	<i>p</i>	<i>r</i>
	3D Thin	3D Thick			
Average tendrils velocity (mm/min)	1.90	1.21	121	.029	.39
Maximum tendrils velocity (mm/min)	12.13	6.65	142	.001	.60
Time of maximum tendrils velocity (%)	83.39	44.60	132	.007	.49
Maximum tendrils aperture (mm)	45.63	37.44	76	.044	.36
Time of maximum tendrils aperture (%)	88.50	79.87	93	.011	.46

Condition 3	Median		<i>W</i>	<i>p</i>	<i>r</i>
	2D Thin	2D Thick			
Average tendrils velocity (mm/min)	1.01	1.10	113	.500	.12
Maximum tendrils velocity (mm/min)	4.70	4.50	107	.341	.34
Time of maximum tendrils velocity (%)	68.10	65.14	121	.371	.16
Maximum tendrils aperture (mm)	55.01	57.03	102	.668	.07
Time of maximum tendrils aperture (%)	83.06	82.24	76	.760	.05

Note. mm = millimetres; min = minutes; 3D = three dimensional; 2D = two dimensional; % = percentage of movement duration.

Condition 2

The results showed that *P. sativum* plants were able to detect the presence of the stimulus in the environment and to modulate the kinematics in term of velocity and the aperture of the tendrils based on different stimulus thickness (Table 4.2. and Fig. 4.3. a and b). The average and the maximum tendrils velocity was higher for the thinner than for the thicker stimulus (Table 4.2.). The maximum tendrils aperture was wider for the thinner than the thicker stimulus (Table 4.2.). Then, the time at which the tendrils reached the maximum velocity and aperture occurred earlier for the thicker compared to the thinner stimulus (Table 4.2.).

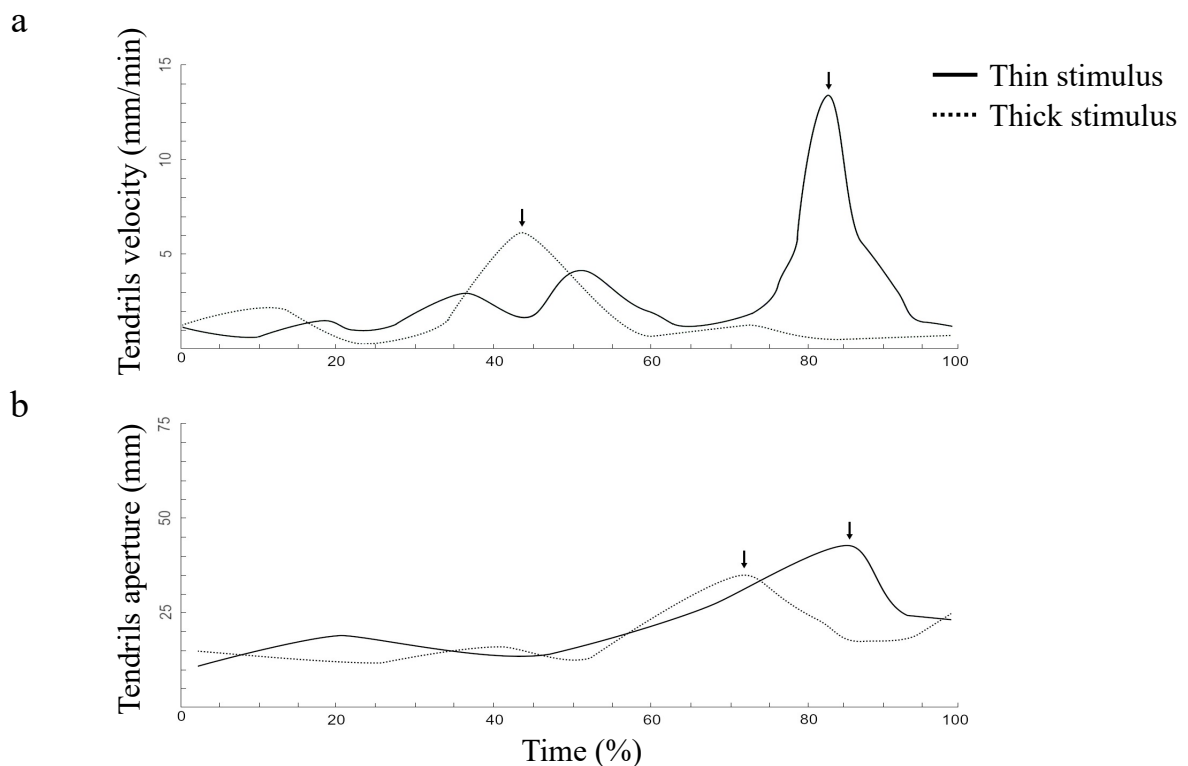


Fig. 4.3. The velocity profile (a) and the tendrils aperture (b) during an approach-to-grasp movements in representative *P. sativum* plants toward a Thin or a Thick stimulus. As highlighted by means of the black arrows the peak velocity and the maximum aperture of the tendrils occurred later for the thinner than the thicker stimulus.

Condition 3

No significant differences for the considered dependent measures across the two conditions (i.e., 2D Thick vs 2D Thin stimulus condition) were found (Table 4.2.).

4.4. Discussion

The present findings show that *P. sativum* plants can perceive an element into the environment and they can modulate their kinematic patterning depending on thickness. In Condition 1 the results showed that when no stimulus (i.e., no stimulus condition) was present plants started to circumnutate to explore the environment searching for a potential stimulus in it and when they failed to detect it, they stopped moving and fell down. However, when plants perceived the presence of the stimulus (i.e., 3D stimulus condition) they knew exactly what to do. Indeed, they rapidly changed the direction of their movement toward the stimulus to approach and clasp it as previously reported (Darwin, 1875; Darwin & Darwin, 1880). Then, quantitative results showed that the kinematics of the approach-to-grasp movement of *P. sativum* plants was affected by the presence/absence of the stimulus in the environment. Indeed, the average and the maximum tendrils velocities was faster for the 3D stimulus condition compared to the no stimulus condition. The maximum tendrils aperture did not differ between the two conditions, but the time of maximum tendrils velocity and aperture did. They occurred earlier for the no stimulus condition compared to the stimulus condition. Results for Condition 1 provide the first 3D kinematical characterization of the approach-to-grasp movement in plants and their ability to plan and execute a “*goal-directed movement*” to satisfy their needs.

Following these results, in Condition 2 I tested whether *P. sativum* plants were also able to modulate their approach-to-grasp behavior based on different stimulus thickness.

Results for Condition 2 pointed out that plants adjusted in flight their approach-to-grasp behavior in term of velocity and the aperture of their tendrils. These results might be discussed in terms of a preference of climbing plants for thinner over thicker supports. Indeed, grasping a support with a greater diameter is a more energy-consuming process than grasping a thinner one (Gianoli, 2015). Several studies have shown that climbing plants are unable to maintain tensional forces and therefore execute a firm grip over thick stimuli (Carrasco-Urra & Gianoli, 2009; Darwin, 1875; Gianoli, 2015; Goriely & Neukirch 2006; Putz, 1984; Putz & Holbrook, 1992). For instance, the twining vines *Wisteria sinensis* (Sims) Sweet and the *Phaseolus coccineus* L. are not able to climb a stimulus with a 15 cm and 8–10 cm in diameter, respectively. Furthermore, ecological studies in forests have reported that the presence of stem twiners is reduced by the presence of trees with a greater diameter (Carrasco-Urra & Gianoli, 2009; Carsten et al., 2002; Putz, 1984; Putz & Chai, 1987). Along these lines, the results outlining a slower movement, a contained aperture of the tendrils, and an anticipated time at which maximum tendrils' aperture occurred might be suggestive of an energy saving strategy for the successful clasping movement for my thicker stimuli.

To better assess whether thickness affects the kinematics of the approach-to-grasp movement in *P. sativum* plants, a third condition was carried out in which movements toward the 2D photographs of the 3D stimuli used for Condition 2 was assessed. The qualitative analysis of the spatial trajectories indicated that the pattern of movement mirrored exactly that observed for the condition in which no stimulus was present in the environment for Condition 1 (Fig. 4.2. a and c). No significant results for all the considered dependent measures between the 2D experimental conditions were reported (Table 4.2.). Therefore, results for Condition 3 showed that the features of the stimulus are fundamental for the planning and the execution of a “goal-directed” movement in plants.

Indeed, when an ungraspable stimulus is detected by the plants, they do not modulate and scale the kinematics of their movement according to stimulus properties (i.e., 2D Thin vs 2D Thick stimulus conditions). In sum, the above results showed that plants might sense and process environmental information and are able to translate these sensory experiences into a complex behavior. Possible speculations regarding how this process might occur will be provided within the “General Discussion”. For the time being I shall report on the characterization of the motoric principles that plants put in place to scale their movement depending on the intrinsic features of the elements they encounter in the environment. In the next chapter I investigate whether in plants there is a tendency for speed to covary with accuracy. An inescapable property reported in a wide range of species, from insects to primates (Burns & Rodd, 2008; Chittka, Skorupski, & Raine, 2009; Ducatez, Audet, & Lefebvre, 2015; Franks, Dornhaus, Fitzsimmons, & Stevens, 2003; Heitz & Schall, 2012).

CHAPTER 5



SPEED-ACCURACY TRADE-OFF

5.1. Introduction

In an ever-changing environment, adaptation is a fundamental process by which an organism modulates its behavior to maintain its fitness. Thus, an efficient system for the processing and the evaluation of the environmental information as to implement a suitable response is necessary. Put simply, an organism should be able to adapt and modulate its movements to deal with the changes occurring in the surrounding.

With this in mind, a successful motor response implies a tendency for speed to covary with accuracy. This phenomenon central to aimed movements is named SAT (Fitts, 1954; Fitts & Peterson, 1964).

SAT has been formalized by Fitts (Fitts, 1954; Fitts & Peterson, 1964). According to Fitts (1954) the human sensory-motor “*channel*” is a limited-capacity system, so that the time to perform an action is proportional to the amount of information required for controlling the movement. This quantity, also known as index of difficulty (ID), depends on the ratio between the size and the distance of the target (Fitts & Peterson, 1964). In particular, according to the Fitts’ law (Fitts, 1954; Fitts & Peterson, 1964), when movements are performed towards targets differing in size (W) and/or positioned at different distances (D), movement time (MT) scales as a logarithmic function of the ratio D/W : $MT = a + b \times \log_2(2D/W)$, with a and $b > 0$ being respectively the intercept and the slope of the regression line, and $\log_2(2D/W)$ representing the ID.

A modern explanation of the Fitts’ effect is provided by the “*optimized-submovement model*” by Meyer and colleagues (1988). According to this model, slower movements

have a smaller scatter of their end-position than faster ones. Undeniably, when the task requires to reach or grasp a smaller or a more distant target, slower movements allow to acquire more information on the target by means of proprioceptive and visual feedback. Using this extra information, any spatial discrepancy between hand and target position is adjusted by implementing corrective adjustments (Meyer, Abrams, Kornblum, Wright & Smith, 1988; Plamondon & Alimi, 1997; this specific issue will be re-taken in Chapter 6).

Aside from humans, SAT mechanisms are at work in a variety of animal species (Chittka et al., 2009). For instance, the motor control of many animals, such as house-hunting ants (Franks et al., 2003), monkeys (Heitz & Schall, 2012), birds (Ducatez et al., 2015) and fish (Burns & Rodd, 2008), is influenced by the size and the distance of the target, as predicted by the Fitts' law.

To date, what remains unknown is whether the Fitts's law applies to brainless organisms such as plants. The findings reported in Chapter 4 makes this a timely and tractable issue. Remember, that I showed that *P. sativum* plants can plan and execute an aimed movement in a flexible and anticipatory manner. That is, plants can explore their environment by means of circumnutation movement to find a potential stimulus and when it is localized, they can modulate the direction and the kinematics of their movement based on different stimulus properties such as its thickness.

Here I capitalize on these results to investigate whether the movement of *P. sativum* plants obeys the Fitts's law. In other words, to assess whether *P. sativum* plants can adjust the velocity of their tendrils during their approach-to-grasp toward stimuli requiring a different level of accuracy.

5.2. Material and methods

The methods are identical to those described in Chapter 3. Exceptions are outlined below.

5.2.1. Sample and growth conditions

Ten healthy-looking *P. sativum* seeds were selected, potted, and grown as specified in Chapter 3 (see Table 5.1.).

Table 5.1. Sample description

	Experimental conditions	
Stimulus	Thin	Thick
N°	5	5
Distance	12 cm	12 cm
Germination period	5 d (\pm 0.8; Range 4 -6)	6 d (\pm 0.8; Range 5 – 7)
Age	15 d (\pm 1.4; Range 10 – 18)	14 d (\pm 1.8; Range 10 – 19)

Note. Germination period and age, which are expressed in days, refer to the median, while median absolute deviation is noted in parentheses.

5.2.2. Experimental conditions

P. sativum plants were tested with a stimulus which was a wooden pole of 60 cm height and of 1.2 cm (Fig. 5.1. a; Thin stimulus condition) or 3 cm (Fig. 5.1. b; Thick stimulus condition) in diameter.

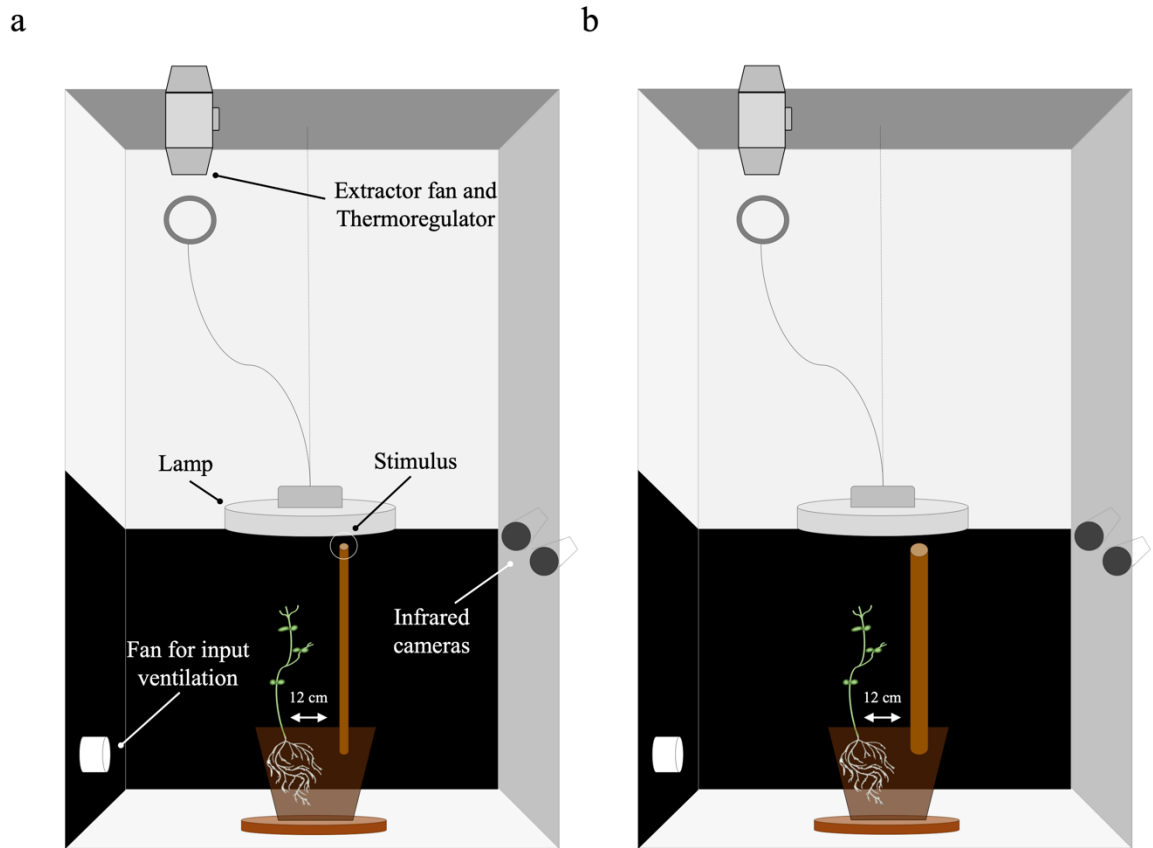


Fig. 5.1. Graphical representation of the experimental setup. (a) The Thin stimulus condition, in which the stimulus was a wooden pole of 60 cm height and of 1.2 cm in diameter. (b) The Thick stimulus condition, in which the stimulus was a wooden pole of 60 cm height and of 3 cm in diameter.

5.2.3. Data recording and processing

In the present study only the tips of the tendrils were considered as the anatomical landmarks of interest and tracked off-line. Two markers were also positioned on the top and the bottom of the stimulus as reference points (Fig. 5.2.).

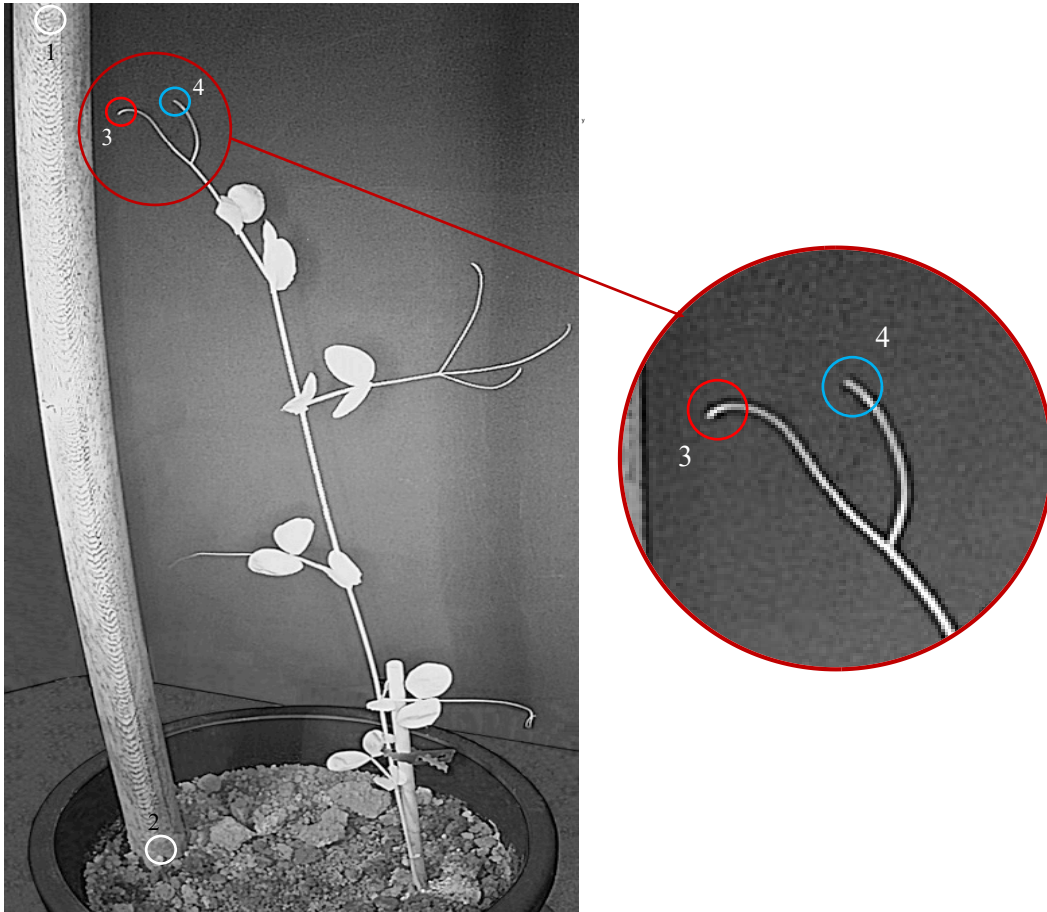


Fig. 5.2. Photograph showing the positioned of the markers on the point of interest: (1,2) the stimulus; (3,4) the tips of the tendrils, which are also showed in the call out.

5.2.4. Dependent measures

The dependent measures specifically tailored to test the experimental hypotheses were (i) the movement time (i.e., the time between the initial frame and the end of plant movement), (ii) the maximum tendrils velocity (i.e., the maximum velocity reached by tendrils during the movement time), and (iii) the average tendrils velocity (i.e., the mean velocity of tendrils during the movement time).

5.2.5. Statistical analysis

Bayesian approach was used to run the statistical analyses, which aims to allocate credibility to a distribution of possible parameter values (i.e., posterior distribution) consistent with the observed data, by generating many samples by using a Markov chain

Monte Carlo method (MCMC). The 95% high-density intervals (95% HDI) are calculated to define which points of the distribution are most credible, and which cover most of the distribution (*see* Kruschke, 2013). A Bayesian t test was used to compare the mean of the tendrils for the Thin (μ_{thin}) and the Thick (μ_{thick}) stimulus conditions. To test it, the BEST (Bayesian ESTimation supersedes the t test) model implemented by Kruschke (2013) allows the calculation of the difference (β) between the mean of the Thin (μ_{thin}) and the Thick (μ_{thick}) stimulus conditions and compared the credibility of $\beta > 0$ with $\beta < 0$. All statistical analyses were performed using the computing environment R (R Core Team, 2014), and the packages BEST (Kruschke & Meredith, 2018) under the default setting.

5.3. Results

5.3.1. Qualitative results

The qualitative results reported that the spatial trajectories of the tendril showed an elliptical pattern of movement during their growth. Then, when the stimulus was detected by the plant the direction of their circumnutating movement veered towards the stimulus to approach and grasp it.

5.3.2. Kinematical results

Movement time

The mean difference of movement time of the tendrils between the Thin ($\mu_{thin} = 2,093 \text{ min} \pm 445$) and the Thick ($\mu_{thick} = 3,082 \text{ min} \pm 1,650$) stimulus condition was $\beta = -989 \text{ mm/min}$, with a 95% uncertainty interval ranging from $-4,230$ to $2,030 \text{ min}$ (Fig. 5.3. a). The probability of $\beta < 0$ (i.e., the probability that the movement time was shorter for the Thin than for the Thick stimulus condition) was 79.3%, whereas the probability of

$\beta > 0$ (i.e., the probability that the movement time was shorter for the Thick stimulus condition) was 20.7%.

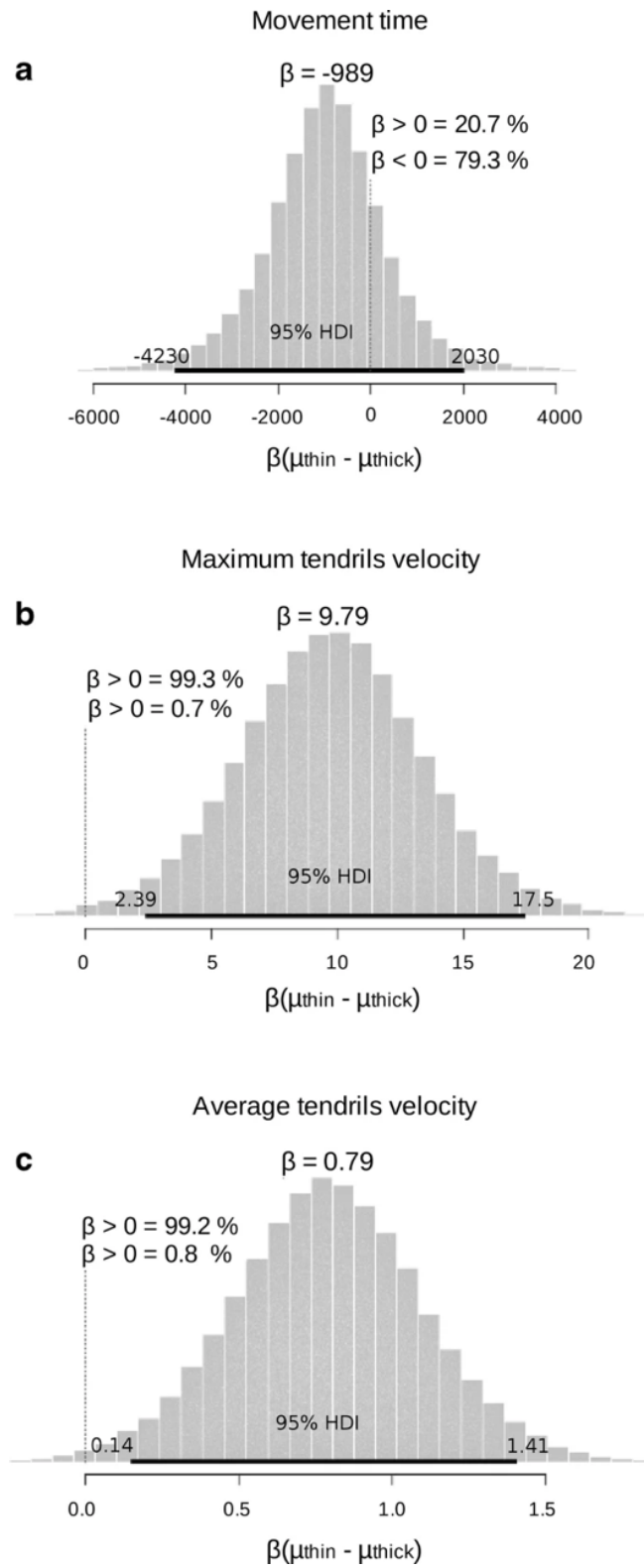


Fig. 5.3. Posterior distributions of β for the movement time (a), the maximum tendrils velocity (b), and the average tendrils velocity (c).

Maximum tendrils velocity

The mean difference of the maximum tendril's velocity between the Thin ($\mu_{thin} = 16.32 \text{ mm/min} \pm 3.70$) and the Thick ($\mu_{thick} = 6.53 \text{ mm/min} \pm 0.91$) stimulus condition was $\beta = 9.79 \text{ mm/min}$, with a 95% uncertainty interval ranging from 2.39 to 17.50 mm/min (Fig. 5.3. b). The probability of $\beta > 0$ (i.e., the probability that the maximum tendrils velocity was larger for the Thin than for the Thick stimulus condition) was 99.3%, whereas the probability of $\beta < 0$ (i.e., the probability that the maximum tendrils velocity was larger for the Thick stimulus condition) was 0.7%

Average tendrils velocity

The mean difference of the average tendril's velocity between the Thin ($\mu_{thin} = 1.88 \text{ mm/min} \pm 0.27$) and the Thick ($\mu_{thick} = 1.09 \text{ mm/min} \pm 0.16$) stimulus condition was $\beta = 0.79 \text{ mm/min}$, with a 95% uncertainty interval ranging from 0.14 to 1.41 mm/min (Fig. 5.3. c). The probability of $\beta > 0$ (i.e., the probability that the average tendrils velocity was larger for the Thin than for the Thick stimulus condition) was 99.2%, whereas the probability of $\beta < 0$ (i.e., the probability that the average tendrils velocity was larger for the Thick stimulus condition) was 0.8.

5.4. Discussion

The present study aimed to assess whether the velocity of the tendrils' movement was modulated as a function of the stimulus's thickness as predicted by the Fitts's law (i.e., the movement of the effector is slower for grasping thinner target than thicker one). The results showed that the plants can sense and process the features of a stimulus and adjust the velocity of their approaching movement with respect to the thickness of the stimulus. However, plants present an opposite pattern with respect of that observed in

different animal species (e.g., Beggs & Howarth, 1972; Fitts, 1954; Heitz & Schall, 2012) in which the velocity of the reaching movement is inversely proportional to ID (i.e., $2D/W$). Here, the average and the maximum tendrils velocity was slower during the approaching and grasping movement toward a thicker stimulus compared to thinner one. Then, the movement time was shorter for the thinner than for the thicker stimulus ($\beta < 0$) with a probability of 79.3%. Therefore, plants consider thick stimulus as a more demanding task with respect to the thin one as previously reported (Gianoli, 2015; Rowe et al., 2006). As already mentioned in Chapter 4, previous findings highlighted that climbing plants have a sort of preference for supports with a smaller diameter given that they tend to produce a lower success rate of attachment over thicker supports compared to thinner ones (Darwin, 1875; Peñalosa, 1982; Putz, 1984; Putz & Holbrook, 1992). Further, it has been demonstrated that the angle between the tip of the tendril and the tangent of the support (i.e., the contact angle) is near-zero value during the coiling phase of the tendrils on stimulus with lower diameter. While the contact angle tends to increase when tendrils must coil around a support with a greater diameter to maintain a firm grip on it (Goriely & Neukirch, 2006). In this view, a slower approaching movement may allow *P. sativum* plants to acquire more evidence on the thick stimulus and to implement corrective adjustments as to reduce the possible risk of errors. Then, the reduction of the velocity of the tendrils' movement may permit to increase the time at which the tendrils encounter the stimulus allowing plants to modulate and correct their trajectories in flight selecting more accurately the contact points to twine around the stimulus firmly.

In sum, these results corroborate the evidence provided in Chapter 4 showing that plants can process the properties of the stimulus before contact and, similarly to animal species, strategically modulate movement velocity according to task difficulty advancing the idea that movement of plants is not only a result of passive reflex to the environmental

stimuli. As already said an attempt to link this evidence with possible sensory and physiological mechanisms will be provided within the “General Discussion”.

However, the present results lead to another question: are plants equipped with a motor accuracy mechanism, which allow them to adjust their movement online and reduce the probability of errors? We know that animals’ species tend to produce corrective adjustments (i.e., secondary submovements) to improve the accuracy of their movements and reduce any spatial discrepancy between the effector and target positions (Ceccarini & Castiello, 2018; Fradet, Lee & Dounskaia, 2008; Novak, Miller & Houk, 2002). Along these lines, in the following chapter (Chapter 6) I will describe a study aimed at assessing whether plants are equipped with a form of movement accuracy mechanism similar of that observed in different animal species, which may allow them to control and adjust their aimed movement by means of secondary submovements and whether this mechanism may be influenced by the difficulty of the task.

CHAPTER 6



MOTOR ACCURACY IN AIMED MOVEMENTS

6.1. Introduction

Previous findings showed that *P. sativum* plants can perceive the stimulus and modulate their tendrils' approach-to-grasp movement based on stimulus thickness (Chapters 4 and 5). Results showed that plants can plan and execute an action which is mediated by action-effect anticipations to satisfy their “wants”. Then, it has been demonstrated that *P. sativum* plants benefit of complex trade-off mechanisms as observed in different animal species (e.g., Chittka et al., 2009; Ducatez et al., 2015; Heitz & Schall, 2012). That is, plants can process the features of the stimulus in flight and adjust the velocity of their tendrils' movement based on the difficulty of the task such as grasping a thick or a thin stimulus (Chapter 5). Above evidence lead to the investigation of another aspect which is important for climbing plants namely the accuracy of the movement which may influence their chances of survival. Therefore, I wonder whether plants are equipped with a motor accuracy mechanism which allow them to adjust their movement online and reduce the probability of errors.

In general, a movement is characterized by two phases: an initial impulse, which concerns the moving of the effector toward the target and the on-line control, which is aimed to adjust the movement improving its accuracy (Ceccarini & Castiello, 2018; Novak et al., 2002). The correction of an aimed movement is given by the production of corrective adjustments namely secondary submovements, which lead to the reduction of any spatial discrepancy between hand and target position (Fradet et al., 2008). In the presence of a difficult task, which requires more precision, more secondary movements

are produced to reduce the end-point variability of an effector, and thus, the probability that the effector fails to grasp the target firmly (Eliasson, Rösblad & Forssberg, 2004; Meyer et al., 1988). Along these lines, here I examine whether climbing plants may have evolved a motor accuracy mechanism as to improve the precision of their movement and how this may differ from those observed in different animal species (Meyer et al., 1988).

To test this, I investigated the approaching movement of *P. sativum* plants toward a thin or a thick stimulus to describe the trajectories of their approach phase and I measured the number of submovements performed in proximity of the stimulus. Furthermore, I considered the endpoint variability (i.e., the variability of tendrils position at the end of the movement) to assess the precision of the movement.

6.2. Material and methods

The methods are identical to those described in Chapter 3. Exceptions are outlined below.

6.2.1. Sample and growth conditions

Ten healthy-looking *P. sativum* seeds were selected, potted, and grown as specified in Chapter 3 (Table 6.1.).

Table 6.1. Sample description

Stimulus	Experimental conditions	
	Thin	Thick
N°	5	5
Distance	12 cm	12 cm
Germination period	6 d (± 0.4 ; Range 4 - 6)	7 d (± 0.8 ; Range 5 - 7)
Age	15 d (± 1.2 ; Range 14 - 21)	22 d (± 4.4 ; Range 10 - 23)

Note. Germination period and age, which are expressed in days, refer to the median, while median absolute deviation is noted in parentheses.

6.2.2. Experimental conditions

P. sativum plants were tested with a stimulus, which was a wooden pole of 60 cm height and of 1.2 cm (i.e., Thin stimulus condition) or 3 cm (i.e., Thick stimulus condition) in diameter (Fig. 6.1. a).

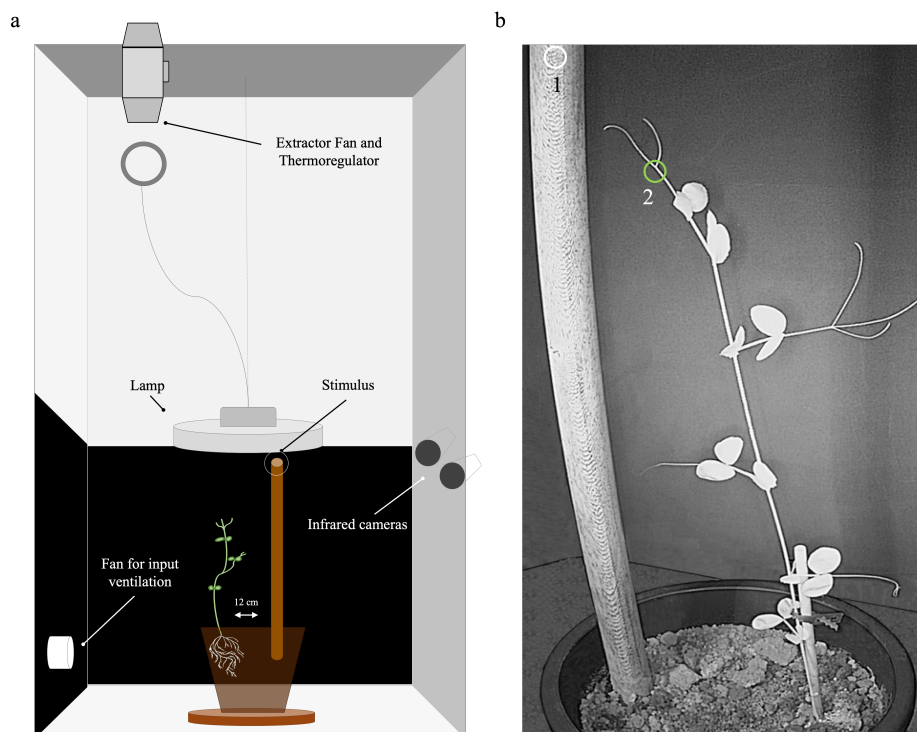


Fig. 6.1. (a) A graphical representation of the experimental setup. (b) A photograph of the position of the markers on the point of interest such as the stimulus (1) and the node below the tendrils (2).

6.2.3. Data recording and processing

In the present study only the node below the tendrils was considered as the anatomical landmarks of interest and tracked off-line. One marker was also positioned on the top of the stimulus and served as a reference point (Fig. 6.1. b)

6.2.4. Dependent measures

The dependent measures specifically tailored to test the experimental hypotheses were the total number of submovements and the endpoint variability. Concerning the total number of submovements, the following submovements traditionally reported in reaching literature (Meyer et al., 1988) were considered: reversals in the trajectory (Type 1 submovement; Fig. 6.2.), defined as a zero crossing from positive to negative value occurred in the velocity profile; re-accelerations toward the target (Type 2 submovement; Fig. 6.2.), defined as a zero-crossing from negative to positive value occurred in the acceleration profile; decreases in the rate of deceleration (Type 3 submovement; Fig. 6.2.), defined as a zero-crossing from positive to negative values appeared in the jerk profile. Given that the corrective adjustments usually emerge near the target (Fradet et al., 2008), secondary movements emerging in the final 5% of the movement time were considered. The endpoint variability of the approaching movement was defined as the standard deviation of the Euclidean distance between the final position of the node below the tendrils and the reference marker located upon the stimulus.

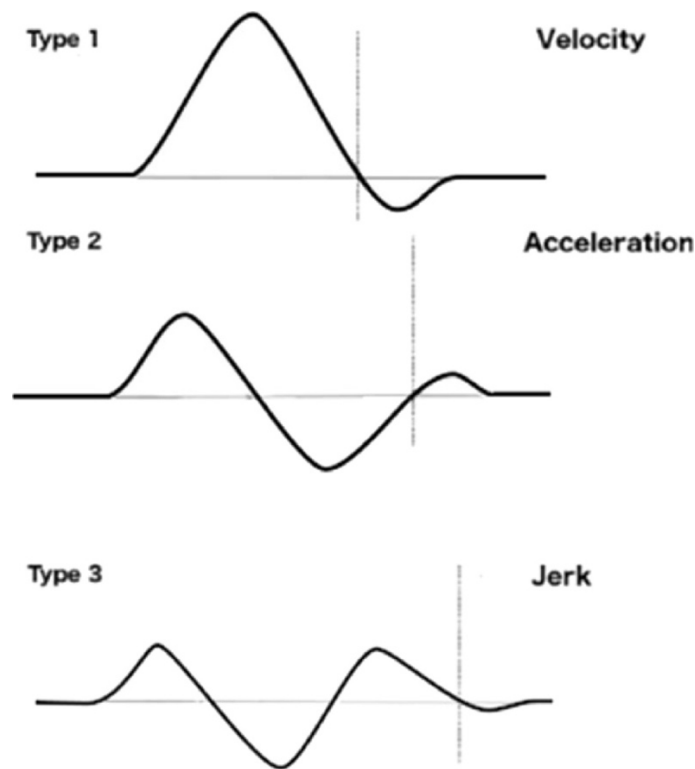


Fig. 6.2. Examples of a discrete movement with secondary submovements of types 1, 2, and 3. The vertical line represents the hypothetical beginning of the secondary submovement.

6.2.5. Statistical analysis

Bayesian approach was used to perform the statistical analyses. This approach reduces the risk of false negatives for small sample size, providing an accurate parameter estimation (Vadillo, Konstantinidis & Shanks, 2016). The total number of the submovements and endpoint variability in both Thin and Thick stimulus conditions were compared using the BEST model, which is implemented by Kruschke (2013). All statistical analyses were performed using the computing environment R and the packages BEST under default setting. The correlation between the total number of submovements and the endpoint variability was computed using `bayes.cor.test` of the package `BayesianFirstAid`.

6.3. Results

6.3.1. Qualitative results

The qualitative results reported that the spatial trajectories of the node below the tendrils for both the Thin and the Thick stimulus conditions showed an elliptical pattern of movement (i.e., circumnutation) during their growth.

6.3.2. Kinematical results

Submovement analysis

The mean difference of the total number of submovements between the Thick ($M = 34.56 \pm 17.35$) and the Thin ($M = 22.46 \pm 5.19$) stimulus condition was 12.10, with a 95% uncertainty interval ranging from -20.60 to 46.10 . The probability that the total number of submovements is larger for the Thick stimulus condition was 82% (Fig. 6.3. a).

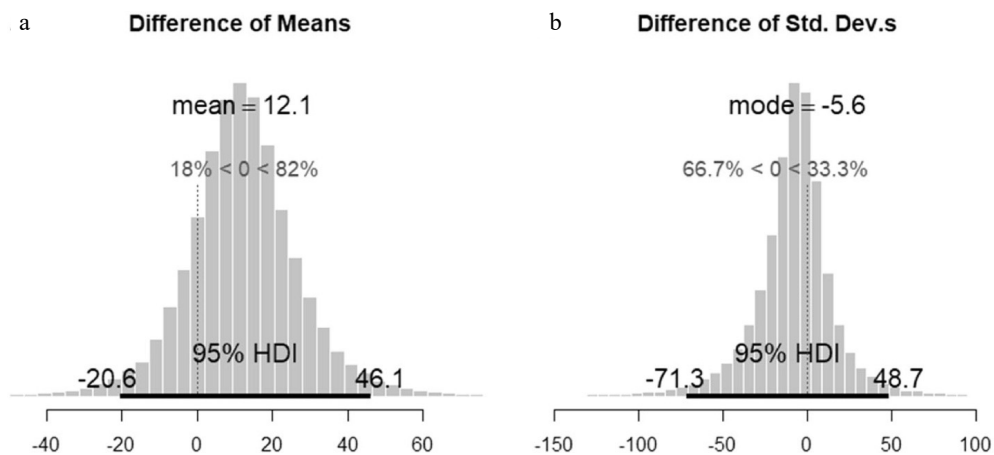


Fig. 6.3. (a) The posterior distributions for total number of submovements. (b) The endpoint variability.

Endpoint variability

The mode differences of the endpoint variability (tendrils position at the end of the movement) between the Thick ($Mo = 17.72$) and the Thin ($Mo = 23.32$) stimulus condition was -5.60 , with a 95% uncertainty interval ranging from -71.30 to 48.70 . The probability

that the endpoint variability is smaller for the Thick Stimulus condition is 66.70% (Fig. 6.3. b).

Correlation analysis

The correlation between secondary submovements and the endpoint variability is -0.26 , with a 95% uncertainty interval ranging from -0.75 to 0.42 . The probability that the endpoint variability is negatively correlated with the total number of submovements is 73.2%.

6.4. Discussion

The present research aimed at assessing whether *P. sativum* plants may correct their approaching movement online by means of secondary submovements and if the production of secondary submovements may be affected by the difficulty of the task. Results showed that plants adjust the trajectory of the node below the tendrils during the approaching phase by modulating the production of submovements as a function of stimulus thickness. Indeed, the frequency of submovements decreases when *P. sativum* plants must approach and grasp a thin stimulus. Then, the analysis of the endpoint variability showed that the standard deviation of the final position of the node below the tendrils is bigger for the thinner than the thicker stimulus. Furthermore, the total number of the submovement is negatively correlated with the endpoint variability suggesting that an increase of the production of the submovements is related with an enhancement of the accuracy of the movement. These results highlighted that *P. sativum* plants can process the features of the stimulus and improve the precision of their movement by using a motor accuracy mechanism.

However, as previously reported in the preceding chapters (Chapters 4 and 5) plants exhibit an opposite pattern of movement than animal species, who experience less difficulty to interact with a thicker target than a thinner one (Fitts, 1954; Fradet et al., 2008). Here, the results showed that *P. sativum* plants tend to produce more secondary submovements when they must approach and grasp a thicker than a thinner stimulus. The plants exhibit more difficulty to grasp a thicker than a thinner stimulus and they tend to implement a more cautionary strategy aimed at coiling the thick stimulus more efficiently. The plants may increase the production of their submovements to reduce the risk of possible errors and to produce a firmer grip on the stimulus. In other words, more adjustments are needed to regulate the twining force and stability of climbing plants for thicker supports (Goriely & Neukirch, 2006; see “General Discussion”). As the grasping success depends on the number of coils around the trellis, thick stimuli will eventually require the development of longer and thicker tendrils, which in turn elicit a greater number of submovements. Moreover, to produce thicker and longer tendrils plants need more energy from photosynthesis as well as to produce more submovements, which are basically fine-tuning changes in cell's growth rates. Then, as more accurate the movement less energy will be expended during the climbing behaviour. This is very important for these plants because often they are living in shadow environments (i.e., lower photosynthesis) fighting to reach more light as to improve photosynthesis. Thus, a cost-benefit “calculation” can be at the basis of movements' changes. Therefore, this adaptation can be interpreted as a trade-off between developing longer and thicker tendrils for grasping thicker stimuli than thinner ones, and a more efficient grasping movement control determined by submovements.

It has been hypothesized that in animal species submovements' production depends on visual and proprioceptive feedbacks of limb position, which are used to make

necessary trajectory corrections (Fradet et al., 2008; Meyer et al., 1988). It should be noted that plants are physically self-aware so that they can perceive the configuration of their own body (Hamant & Moullia, 2016; Karban, 2015). Recent reports demonstrate that the proprioception of plants is mediated by the long actin filaments in elongating fibre cells, that, acting as a bending tensile sensor, perceive the plant's posture (Karban, 2015). These findings are corroborated by studies on *A. thaliana* mutants defective in actins (specifically ACTIN-8), which exhibit an abnormal reaction in response to gravity, tilting or other external perturbations (Hamant & Moullia, 2016). Proprioception may allow climbing plants to know the position of their tendrils and contribute to generate the necessary feedback information required for adjusting movement execution working in tandem with other sensory modalities (see "General Discussion").

In sum, the present results corroborate the idea that even though plants are stuck in the ground they might plan and control their movement in a very flexible and accurate manner.

CHAPTER 7



THE CODING OF STIMULUS THICKNESS: DO ROOTS MATTER?

7.1. Introduction

The findings reported in the previous chapters (Chapters 4, 5 and 6) strongly suggest that plants can extract the “*graspable*” properties of a stimulus that permits them to select the most opportune movement and behavior to reach more light. In other words, climbing plants seem to plan actions in terms of their perceivable consequences such as selecting, planning, and initiating, suggesting that such activities are mediated by action-effects anticipation (Calvo & Friston, 2017). This leads to the hypothesis that plants may be endowed with anticipatory behavior.

As already anticipated, the mechanisms underlying thickness coding by plants remain obscure (Chapters 4, 5 and 6). And as I shall outline within the “General Discussion” section a variety of hypotheses based on plants’ exceptional perception abilities have been put forward.

Interestingly, as you will read, most of these hypotheses seem somewhat linked to the aerial sector of plants. However, mounting evidence suggests that the roots and in particular their extreme tip (i.e., root cap) may also be involved in the processing of numerous signals (Baluška et al., 2004; 2009; 2010) assess them, and dynamically control the direction of root growth (Hammond & White, 2011; Trewavas, 2017).

A great deal of plant decision-making can be ascribed to the root cap system (Baluska et al., 2004, 2009; 2010). Consider, for instance, the highly sophisticated

responses of roots, such as gravitropisms and thigmotropism (Baluška, Barlow, Volkmann & Mancuso, 2007; Braam, 2005). Moreover, roots stop developing downward when they encounter a physical obstacle and instead begin to grow horizontally. And they seem to be able to respond to stimuli and periodically move downward, remaining horizontal if unable to respond gravitropically (Massa & Gilroy, 2003). Roots have also shown salt-avoidance behavior (Li & Zhang, 2008). As salinity interacts with the gravitropic response of shoots, plants are forced to assess the overall integrated signal to optimize shoot growth under abnormal saline conditions (Barlow, 2010; Trewavas, 2005).

It is, however, still unknown the signals that are chiefly involved in obstacle perception, despite the touch sensor appears to be the most likely candidate given its immediate physical interaction with the obstacle (Darwin & Darwin, 1880). It is also unknown which component(s) or organelles in the root cells are utilized to perceive a mechanical force, and how they convert a physical touch signal to the auxin system. Auxins are a class of plant hormones that play a cardinal role in coordinating many growths and behavioral processes in plant life cycles and they are essential for plant body development. There is also evidence that obstacle avoidance may rely on root exudates, the cocktail of compounds that are secreted by roots, at the root cap (Semchenko et al., 2008). Root recognition and navigation around physical obstacles is in fact prevented when activated charcoal, which absorbs many compounds, is inserted into the substrate (Mahall & Callaway, 1992).

In the light of these considerations, the research question addressed by the current study is: are roots able to sense the thickness of a potentially available stimulus? To test this, in the footsteps of my previous studies (Chapters 4, 5 and 6), I performed two experiments. In Experiment 1, I set out to replicate the kinematical effects observed when stimuli of different thicknesses are available to the root system (Chapters 4 and 5). One

group of plants was tested with a Thick stimulus (i.e., 3 cm diameter), another with a Thin one (i.e., 1.2 cm diameter). In line with previous findings (Chapters 4 and 5), I expected the peaks of the average and maximum velocities of the tendrils to be higher for the thinner stimulus with respect to the thicker one and the times it took the tendrils to reach the peak velocity and the maximum aperture to be later for the thinner stimulus with respect to the thicker stimulus. Finally, I expected the maximum distance between the tendrils to be significantly greater for the thinner stimulus with respect to the thicker stimulus. Experiment 2 was a replication of Experiment 1 except that the stimulus was lifted to the ground by means of an ad hoc apparatus. This manipulation allowed investigating the pattern of tendrils movement when the stimulus is unavailable to the root system. I hypothesized that (a) if the root system plays a pivotal role in determining tendril shaping during growth, then kinematical scaling of the velocity and aperture of the tendrils depending on the thickness of a stimulus would be affected when the stimulus is not in the ground because that information is not available to the root system; (b) if the root system does not play a pivotal role in determining the tendril shaping during growth, then the kinematical scaling depending on the thickness of a stimulus should take place even in the case in which the stimulus is unavailable to the root system as thickness information could be coded by the aerial components of the plant (e.g., tendrils or the apex).

7.2. Material and methods

For both experiments the methods are identical to those described in Chapter 3. Exceptions are outlined below.

7.2.1. Sample and growth conditions

Ten (Experiment 1) and twenty-one (Experiment 2) healthy-looking *P. sativum* seeds were selected, potted, and grown as specified in Chapter 3 (Table 7.1).

Table 7.1. Sample description

Experiment 1		
Stimulus	Thin	Thick
N°	5	5
Distance	12 cm	12 cm
Germination period	6 d (\pm 0.4; Range 4 - 6)	6 d (\pm 1.6; Range 5 – 10)
Age	15 d (\pm 3.2; Range 14 – 26)	22 d (\pm 1.4; Range 19 – 23)
Experiment 2		
Stimulus	Thin	Thick
N°	11	10
Distance	12 cm	12 cm
Germination period	6 d (\pm 1; Range 4 - 8)	5.5 d (\pm 1.5; Range 4 – 9)
Age	18 d (\pm 4; Range 14 – 37)	19.5 d (\pm 6; Range 11 – 31)

Note. Germination period and age, which are expressed in days, refer to the median, while median absolute deviation is noted in parentheses.

7.2.2. Experiments

Experiment 1

P. sativum plants were tested with a stimulus, which was a wooden pole of 60 cm height and of 1.2 cm (i.e., Thin stimulus condition) or 3 cm (i.e., Thick stimulus condition) in diameter (Fig. 7.1. a).

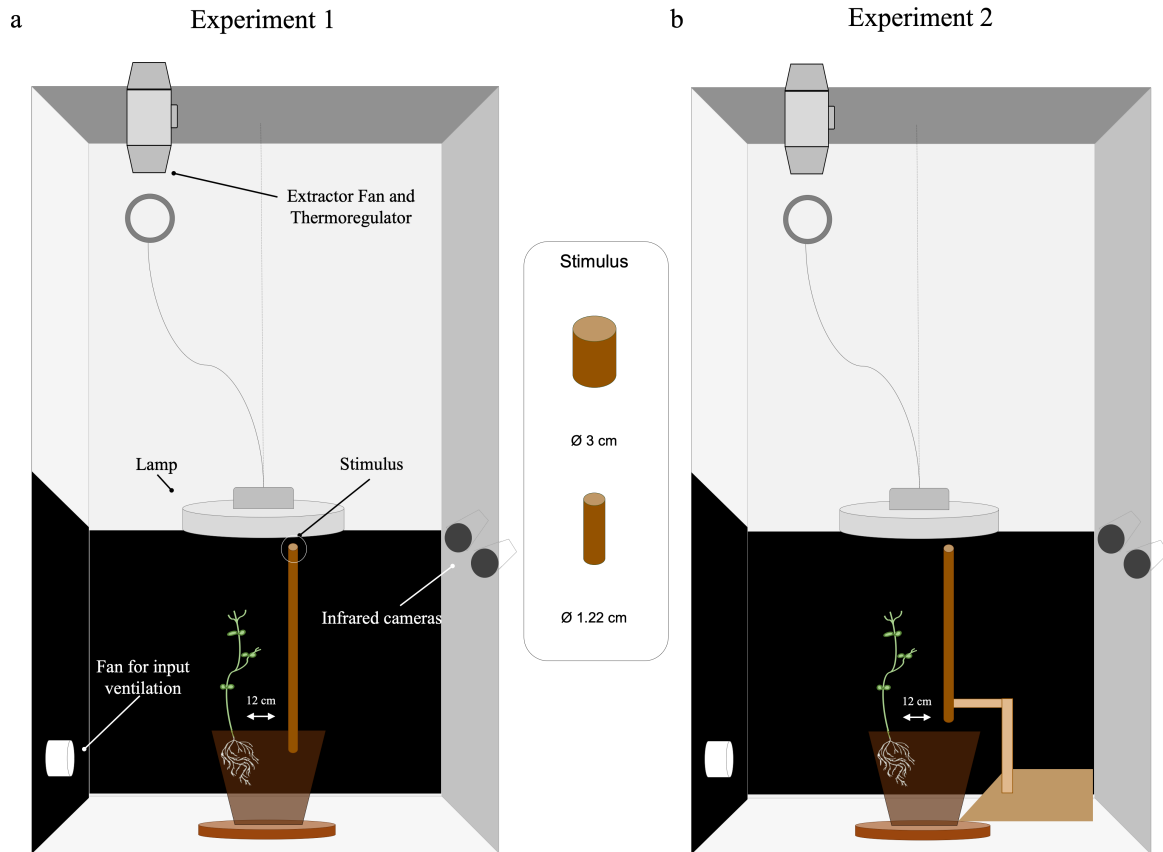


Fig. 7.1. (a) Graphical representation of the experimental setup. (a) Experiment 1 in which the stimulus was either a wooden pole of 60 cm height and of 1.2 cm or 3 cm in diameter. (b) Experiment 2, in which the stimulus was a similar stimulus used in the Experiment 1, but it was raised to the ground by means of an ad hoc apparatus.

Experiment 2

P. sativum plants were tested with a similar stimulus used in the Experiment 1, but it was raised to the ground by means of an ad hoc apparatus (Fig. 7.1. b). The stimulus was positioned at 12 cm in front of the first unifoliate leaf for each plant.

7.3. Results

7.3.1. Qualitative results

All plant organs (i.e., the apex, the node below the tendrils, and the tendrils; Fig. 7.2. a) presented a growing pattern of movement which was characterized by circular or elliptical movement around the central axis of the plant (Fig. 7.2. b and c). In Experiment

1, *P. sativum* plants started to circumnutate to explore the environment searching a potential stimulus in it, and when they detected the stimulus plants changed the trajectories of their tendrils' movements to approach and grasp it (Fig. 7.2. b and c). A similar behavior was observed also in Experiment 2 but only when the tendrils encounter the stimulus accidentally. In this case, *P. sativum* plants modified their movement trajectories toward the stimulus to clasp it. Importantly, this pattern of movement was observed only for the thick stimulus (Fig. 7.2. b). When the lifted stimulus was thin, plants continued to circumnutate and move toward the light source because, given the reduced dimension of the stimulus, it might be impossible for plants to touch it accidentally (Fig. 7.2. c).

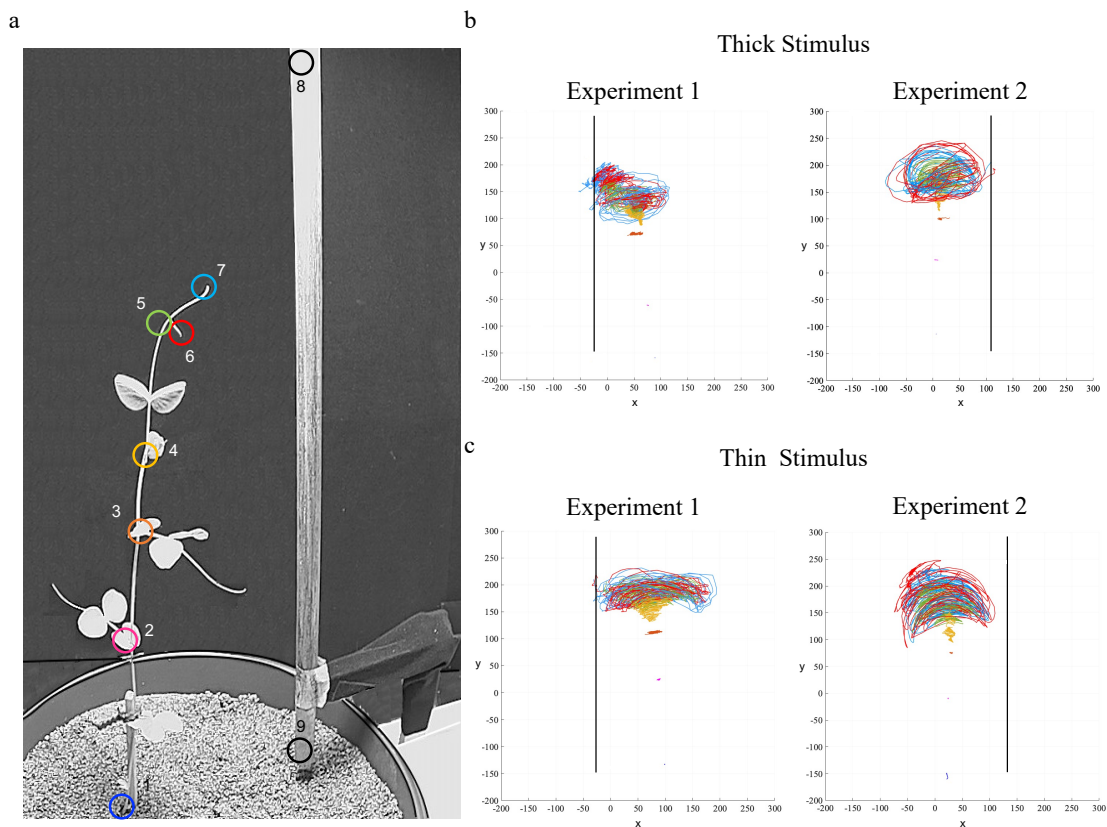


Fig. 7.2. (a) Photograph showing the position of the markers on the point of interest: (1) the origin point of the plant; (2) the second leaf; (3) the internode; (4) the apex; (5) the node below the tendrils; (6,7) the tip of the tendrils; (8,9) the stimulus. The colors of the circles correspond to the colors of the trajectories shown in panel b and c. (b) The trajectories for all the landmarks for the Thick Stimulus in Experiments 1 and 2 are shown. (c) The trajectories for all the landmarks for the Thin Stimulus in Experiments 1 and 2 are shown.

7.3.2. Kinematical results

Experiment 1

When the stimulus was grounded and so it was available to the root system, results showed that the kinematics of the approach-to-grasp movement in *P. sativum* plants was affected depending on the thickness of the stimulus in the environment (Table 7.2.). As previously reported, the average and the maximum tendril velocity were significantly higher for the thinner with respect to the thicker conditions (Table 7.2.). The time at which the maximum tendril velocity was reached earlier for the thicker than the thinner condition (Table 7.2.). The maximum tendril aperture, corresponding to the maximum distance reached by the tips of the tendrils during the approach phase, was wider for the thinner than the thicker condition (Table 7.2.). The time at which the maximum distance was reached was earlier for the thicker than the thinner condition (Table 7.2.). When the roots can access the stimulus, the plants are able to perceive it and to plan movements depending on its thickness as previously reported (*see* Chapters 4 and 5).

Experiment 2

When the stimulus was not available to the root system, the plant was unable to perceive it or to plan movements appropriate to the stimulus' thickness (see Table 7.2.).

Table 7.2.

Kinematical and statistical values for Experiments 1 and 2

Experiment 1					
Thick vs Thin Stimulus	Median		<i>W</i>	<i>p</i>	<i>r</i>
	Thick	Thin			
Average tendril velocity (mm/min)	1.24	2.91	200	0.002	0.69
Maximum tendril velocity (mm/min)	5.24	17.25	218	0.001	0.73
Time of maximum tendril velocity (%)	64.21	90.2	156	0.037	0.38
Maximum tendril aperture (mm)	51.31	76.83	161	0.022	0.41
Time of maximum tendril aperture (%)	41.61	76.32	156	0.037	0.38
Experiment 2					
Thick vs Thin Stimulus	Median		<i>W</i>	<i>p</i>	<i>r</i>
	Thick	Thin			
Average tendrils velocity (mm/min)	2.38	2.23	798	0.839	0.02
Maximum tendril velocity (mm/min)	10.62	11.75	739	0.449	0.08
Time of maximum tendril velocity (%)	39.40	47.29	814	0.958	0.01
Maximum tendril aperture (mm)	42.20	48.20	311	0.135	0.20
Time of maximum tendril aperture (%)	58.84	88.58	359	0.467	0.10

Note. mm = millimeters; min = minutes; % = percentage of movement duration.

7.4. Discussion

The current study set out to investigate if the root system contributes to perceive and respond to stimuli of different thickness. The results indicate that if the roots do not have access to the stimulus, the plant does not modulate its tendrils' response to different thicknesses.

The key functions of the roots are to acquire nutrients and water and to provide anchorage for the plant. Indeed, the vast majority of studies have focused on foraging

strategies by the roots and their behavioral responses to multiple environmental cues such as the heterogeneous soil environment and the presence of other factors such as competitors (reviewed by Cahill & McNickle, 2011; Hodge, 2004; 2009). Roots' ability to monitor and integrate numerous parameters simultaneously and to “*translate*” these sensory “*experiences*” into complex motoric responses has been described (Gandar, 1983; Massa & Girloy, 2003; Semchenko et al., 2008). Charles and his son Francis Darwin (1880) observed that roots tend to avoid inanimate obstacles, a behavior that is more dominant than the root's tendency to develop geotropically (Darwin & Darwin, 1880). They also noted that when the tip of *V. faba* roots came into contact with a thin glass plate at a steep angle, it modified its morphology and growth direction. Another similar example refers to the tip of the radicle which can distinguish between harder and softer objects pressed upon it. If the tip perceives the air to be moister on one of the sides, it transmits the information to the upper adjoining part, which bends towards the source of moisture. In almost every case, the movements reported seemed to be driven by a goal (Darwin & Darwin, 1880). In other words, the fate and the morphology of roots seem to be determined by complex interactions with various environmental factors such as potential physical obstructions and objects in the soil. When they make contact with physical obstacles, roots tend to grow toward areas of least resistance (Clark et al., 2003; Kozłowski, 1999). Circumventing physical obstacles could be critical for the plant's survival and performance, especially in rocky environments or when the soil is compacted. Wilson (1967), who was one of the first to investigate the pattern of woody root growth in maple trees in the presence of artificial barriers, showed that tree roots tended to return to their original growth point when they detected an obstacle. It has subsequently, been demonstrated that in *A. thaliana* there are six genes involved in the stimulus-response interaction. In other words, the interaction between obstacles and the root cap affects the

geotropic response which eventually reorients the roots leading them to grow along obstacles (Okada & Shimura, 1990).

Although it has long been known that roots have the ability to circumvent obstacles (Darwin & Darwin, 1880; Montagu, Conroy & Francis, 1998; Simojoki, 2001), the mechanism underlying the obstacle avoidance response by roots is poorly understood. The root bending response, which does not seem to be just a passive response to an obstacle, appears to depend on a complex process. Evidence suggests that root obstacle avoidance is driven by the accumulation of root exudates at the root cap (Semchenko et al., 2008). It has recently been demonstrated that the bending of a root away from an obstacle involves active signal transduction and depends on both the PIN-mediated auxin transport (PIN auxin) and the Transport Inhibitor Response1/Auxin Signaling F-box protein (TIR1/AFB) auxin signaling pathway (Lee, Kim, Park, Cho & Jeon, 2020; Zhang & Friml, 2020). Other findings have confirmed that physical obstruction induces changes in the placement of root mass such as fewer and lower lateral roots not noted in areas without obstacles (Hodge, 2009; Semchenko et al., 2008).

Whether roots are also able to process the intrinsic features of the objects they encounter has received little attention in ecological and physiological literature. In this respect, the results outlined here not only confirm that the roots of *P. sativum* plants are sensitive to inanimate objects (e.g., Takahashi & Suge, 1991), but also that such ability extends to the coding of structural characteristics (i.e., thickness).

It has already been demonstrated that *P. sativum* plants are able to detect and avoid inanimate objects in the soil (Falik et al., 2005). This effect appeared to be mediated by the diffusion and accumulation of root exudates in the vicinity of the objects, causing inhibited root growth in the direction of the accumulated exudates (Falik et al., 2005). It has been experimentally proven that plants do not perceive objects and even grow towards

them if exudates have been removed from the substrate (Falik et al., 2005). This mechanism might also explain the present results. The diffusion and accumulation of root exudates serve not only to avoid growth in the vicinity of inanimate objects (Darwin & Darwin, 1880; Falik et al., 2005; Leyser & Day, 2003) but also to process the features of the objects encountered. In other words, diffusion properties, accumulation and effectiveness of exudates may signal the physical properties of a potential stimulus. Although study findings have not identified the substances that are responsible for the ability to process object thickness, they suggest that chemical compounds might be involved to this endeavor.

To conclude these findings support the idea that the root tip functions are capable of processing multiple environmental cues providing information about the underground environment and leading to adaptive behavior. The idea is in line with the “*root-brain hypothesis*” of Charles and Francis Darwin (1880) according to which each root apex acts as both a sensory organ and a “*brain-like*” command center generating specific cognition and behavior (Baluška et al., 2004; 2009; 2010). Roots, thus, may be able to solve cognitive problems such as where, how or whether to grow at all and to fill the interaction between effectors and objects (Castiello, 2020; Hodge, Berta, Doussan, Merchan & Crespi, 2009; Trewavas, 2009). These issues will be re-taken within the “General Discussion” section. In the next chapter I shall further explore the “*roots-brain hypothesis*” with an eye on the crosstalk between the roots and the aerial component of the plants.

CHAPTER 8



ROOT-TO-SHOOT SIGNALLING IN STIMULUS THICKNESS CODING

8.1. Introduction

In the previous study (Chapter 7), it has been demonstrated the pivotal importance of the root system for the coding of stimulus thickness in *P. sativum* plants. Indeed, it has been observed that when the root system does not have access to the stimulus because it was lifted to the ground, the plant does not modulate its tendrils' response to different thicknesses. Whereas, when the stimulus was available to the roots, the plants were able to adjust the kinematic of their tendril's movement in term of velocity and aperture depending on different stimulus thickness as previously reported (Chapters 4, 5 and 7). These results suggest that the root system is involved in sensing the presence and the thickness of a stimulus and that the information perceived affects the planning and the execution of the climbing plants' approach-to-grasp movements. At least they suggest that the aerial components of the plants (i.e., stem and tendrils) alone are unable to code for the thickness of the stimulus. This is an intriguing and puzzling conclusion that inevitably calls for a question: why does the *P. sativum* plants rely on underground stimuli to drive aboveground behaviour? Indeed, what the root system finds in the soil might not be a reliable proxy for what is happening above it.

In the light of all these considerations, the research question addressed by the current study is to further investigate the contribution of the root system to the coding of stimulus thickness by exploring the functional equilibrium and interactivity between the root system and the shoot growth (Brouwer, 1963).

To this end, the movement of *P. sativum* plants towards a stimulus that differed in thickness with respect to the above- and belowground parts was assessed (i.e., perturbed conditions). A group of plants was tested with a stimulus in which the belowground part was thin, and the aboveground part was thick (i.e., Thin-Below condition; see Fig. 8.1. a); and another group was tested with the inversed conditions: the stimulus was thick belowground and thin aboveground (i.e., Thick-Below condition; see Fig. 8.1. b). Movements during perturbed trials were compared with one-sized stimuli that could be either thin or thick (i.e., control conditions). Specifically, trials for the Thin-Below perturbed condition were compared with trials for the Control-Thick condition (Fig. 8.1. a and d), and movements for the Thick-Below condition were compared with movements for the Control-Thin condition (Fig. 8.1. b and c).

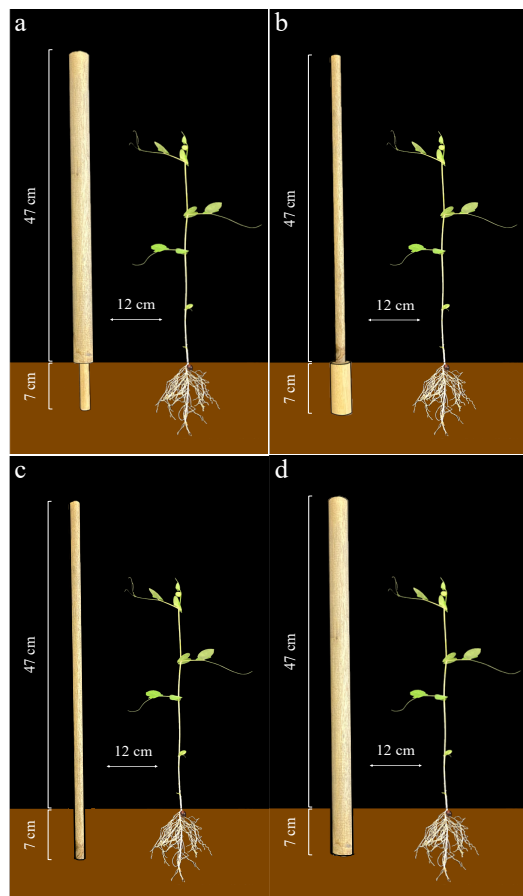


Fig. 8.1. The experimental stimuli. (a) Stimulus for the Thin-Below condition. (b) Stimulus for the Thick-Below condition. (c) Stimulus for the Control-Thin condition. (d) Stimulus for the Control-Thick condition.

It has been hypothesized that if the roots play a pivotal role in sensing stimulus thickness, then kinematical parameterization would be driven by the thickness of the belowground part of the stimulus. In such circumstances, I expect differences between the perturbed and the control conditions because the thickness for the belowground part of the stimulus used for the perturbed conditions differs from the thickness of the stimulus used for the control conditions. Remember that the movement of *P. sativum* plants towards stimuli of different thicknesses are characterized by specific kinematical signatures (Chapters 4, 5 and 7).

On the other hand, if the aerial part of the plant has some involvement in sensing stimulus thickness, then no significant differences between the perturbed and control conditions should be observed given that the thickness for the upper part of the stimuli used for the perturbed conditions is similar to the stimuli used for the control conditions.

A third hypothesis implies a crosstalk between the roots and the aerial part of the plant that inevitably will be affected by the mismatch between the below- and aboveground part of the stimuli. If the belowground information is incongruent with the end-goal of the movement, an adjustment by the aboveground organs (i.e., stem, tendrils, ...) should occur and the movement reprogrammed accordingly.

8.2. Material and methods

The methods are identical to those described in Chapter 3 except for what follows.

8.2.1. Sample and growth conditions

Forty healthy-looking *P. sativum* seeds were selected (Table 8.1.), potted, and grown as specified within the “*Sample Description*” section of Chapter 3.

Table 8.1. Sample description

Control-Thick vs Thin-Below		
	Control-Thick	Thin-Below
N°	10	10
Distance	12 cm	12 cm
Germination period	6 d (\pm 0.5; Range 4 – 10)	5 d (\pm 1.22; Range 5 – 12)
Age	21 d (\pm 3.1; Range 14 – 26)	16.5 d (\pm 1.7; Range 14 – 19)
Control-Thin vs Thick-Below		
	Control-Thin	Thick-Below
N°	10	10
Distance	12 cm	12 cm
Germination period	5.5 d (\pm 0.6; Range 4 – 7)	5 d (\pm 1.5; Range 3 – 10)
Age	14 d (\pm 2; Range 10 – 20)	21.5 d (\pm 5.6; Range 9 – 26)

Note. Germination period and age, which are expressed in days, refer to the median, while median absolute deviation is noted in parentheses.

8.2.2. Experimental conditions

The stimuli were 54 cm in height wooden poles (the belowground part of the stimulus was 7 cm in height, while the aboveground part of the stimulus was 47 cm height).

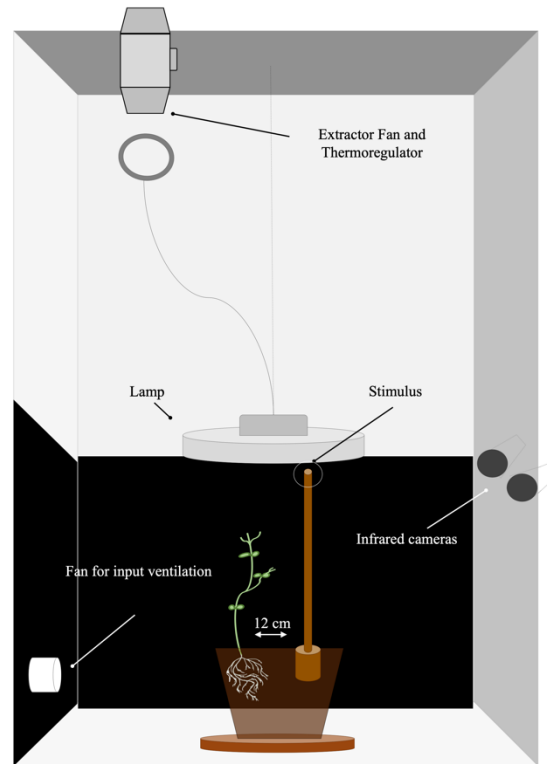


Fig. 8.2. Graphical representation of the experimental set up. The represented example is for the Thick-Below condition.

The stimulus varied in diameter depending on experimental conditions (see Fig. 8.1.). For the (i) Thin-Below perturbation (Fig. 8.1. a) the belowground part of the stimulus was 1.2 cm in diameter, whereas the aboveground part of the stimulus was 3 cm in diameter; (ii) Thick-Below perturbation (Fig. 8.1. b), the belowground part of the stimulus was 3 cm in diameter, whereas the aboveground of the stimulus was 1.2 cm, (iii) Control-Thin condition (Fig. 8.1. c) the diameter was 1.2 cm; (iv) Control-Thick condition (Fig. 8.1. d) the diameter was 3 cm in diameter.

8.3. Results

8.3.1. Qualitative results

For all the experimental conditions, the tip of the tendrils showed a growing movement pattern characterized as circumnutation (Fig. 8.3. a and b), which aims to find a

potential stimulus in the environment. Once the plant detected and perceived the stimulus, strategically modified the trajectory of its tendrils that started to bend toward the stimulus to approach and clasp it. Importantly, plants directed their movement toward the stimulus and shaped the choreography of the tendrils depending on stimulus thickness before any physical contact with it (Chapters 4, 5 and 7).

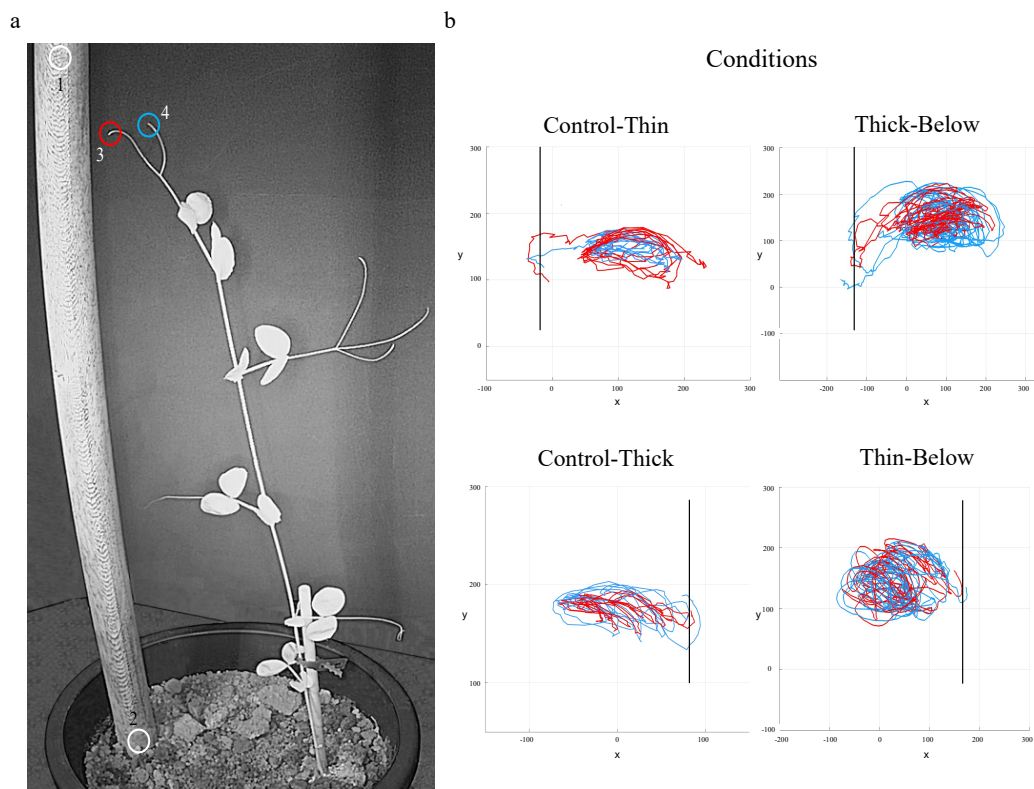


Fig. 8.3. The landmarks considered and examples of the spatial trajectories. (a) The landmarks considered were the stimulus (1, 2) and the tip of the tendrils (3, 4). The colours of the circles correspond to the colours of the trajectories shown in the right-side panel. Panel (b) represents the trajectories for the tendrils for the Control-Thin, Thick-Below, Control-Thick and Thin-Below stimulus conditions. The stimulus is represented with a solid line. The axis x and y refer to the sagittal and vertical axis in mm, respectively.

8.3.2. Kinematical results

Control conditions

The pattern of results obtained for the control conditions mirrors those reported in previous studies in which one-sized thick and thin stimuli were compared (see Table 2;

Chapters 4, 5 and 7). As shown in Fig. 8.4. movement time was longer for the Control-Thick than the Control-Thin condition. The maximum tendrils velocity was lower, and the peak of maximum tendrils velocity occurred earlier for the thicker than the thinner stimulus (Table 2). The maximum tendrils aperture was wider and the time at which it occurred was later for the thinner than the thicker stimulus (Table 2). This aspect is important because it provides a confirmation that *P. sativum* plants exhibit a different kinematical pattern for thick and thin stimuli (Chapters 4, 5 and 7). And therefore, they provide the ideal comparison for investigating the effects determined by the perturbed conditions.

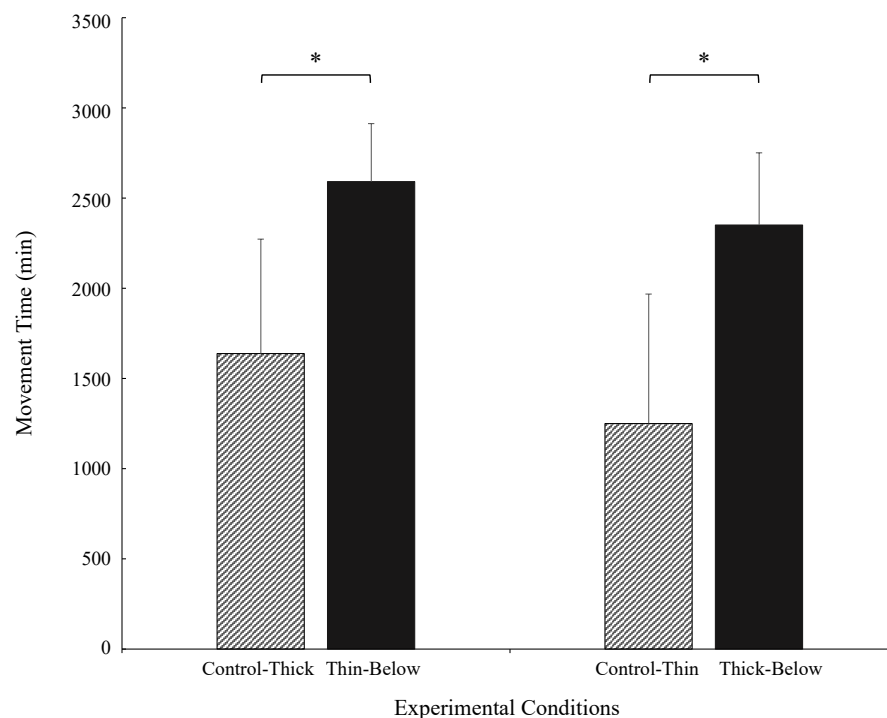


Fig. 8.4. Bar plots representing the kinematical values for the comparisons between the Control-Thick and Thin-Below stimulus conditions and between the Control-Thin and Thick-Below stimulus conditions for the Movement time. Bars refer to the median, while error bars refer to the absolute deviation (MAD). Asterisks indicate that the difference between the perturbed and the control conditions were significant.

Thin-Below vs Control-Thick

Movement time was longer for the perturbed than for the control condition ($W=297$; $p=.024$; $r=.29$; Fig. 8.4.). The maximum tendrils velocity was higher for the perturbed than for the control condition ($W= 192$; $p=.001$; $r= .41$; Table 2). The time at

which the maximum velocity of the tendrils occurred was earlier for the perturbed than for the control condition ($W= 540$; $p=.006$; $r= .35$; Table 2). The maximum aperture of the tendrils was greater for the perturbed than for the control condition ($W= 134$; $p<.001$; $r=.46$; Table 2). Further, the time at which the maximum aperture of the tendrils occurred was earlier for the perturbed than for the control conditions ($W= 460$; $p=.004$; $r= .37$; Table 2).

Thick-Below vs Control-Thin

Movement time was longer for the perturbed than for the control condition ($W=238.5$; $p=.001$; $r=.40$; Fig. 8.4.). Similarly, the peak of maximum velocity of the tendrils occurred earlier for the perturbed than for the control trials ($W= 483$; $p=.037$; $r=.27$; Table 2).

Table 2
Kinematical values

Control-Thick vs Control-Thin	Median	
	Control-Thick	Control-Thin
Maximum tendrils velocity (mm/min)	11.86	13.04
Time of maximum tendrils velocity (%)	76.78	79.93
Maximum tendrils aperture (mm)	30.80	38.52
Time of maximum tendrils aperture (%)	81.9	87.22
Control-Thick vs Thin-Below	Median	
	Control-Thick	Thin-Below
Maximum tendrils velocity (mm/min)	11.86	15.05
Time of maximum tendrils velocity (%)	76.78	46.09
Maximum tendrils aperture (mm)	30.80	53.40
Time of maximum tendrils aperture (%)	81.9	68.72
Control-Thin vs Thick-Below	Median	
	Control-Thin	Thick-Below
Maximum tendrils velocity (mm/min)	13.04	14.36
Time of maximum tendrils velocity (%)	79.93	55.37
Maximum tendrils aperture (mm)	38.52	42.66
Time of maximum tendrils aperture (%)	87.22	84.56

Note. mm = millimeters; min = minutes; % = percentage of movement duration.

8.4. Discussion

Here, the interplay between the below- and aboveground parts of *P. sativum* plants for the coding of stimulus thickness were explored. The results show differences in kinematical patterning when comparing perturbed and control trials. First and foremost, for both the perturbed conditions movement duration is longer than for the control trials,

suggesting that the thickness mismatch characterizing the perturbation reflects on the duration of the movement. It might well be that the more information is needed to complete a task, the higher the time to perform it. Indeed, the perturbed stimulus requires more processing than the unperturbed stimulus given that the evaluation for the two thicknesses needs to be performed.

When comparing the Thin-Below with the Control-Thick conditions, it is observed a differential pattern of results depending on the considered kinematical measures. The amplitude of maximum velocity and aperture of the tendrils suggest that *P. sativum* plants adjust the kinematical pattern of the movement on the basis of the belowground (i.e., thin) part of the stimulus. That is, the maximum tendrils velocity was higher, and the maximum tendrils aperture was wider for the perturbed than for the Control-Thick condition.

Remember that this is the pattern previously observed for one-sized thinner stimuli (Chapters 4, 5 and 7). Therefore, it seems that the pattern of the movement in terms of amplitudes is based on the information provided by the root system. However, when considering the temporal parameters, the time at which the maximum peak velocity and grip aperture occurred, *P. sativum* plants adjust the kinematical pattern of the movement on the basis of the aboveground (i.e., thick) part of the stimulus. That is, the time at which the peaks of maximum velocity and maximum aperture of the tendrils occurred it was earlier for the perturbed than for the control condition. Remember that this is the pattern previously observed for one-sized thick stimuli (Chapters 4, 5 and 7). Consequently, it seems that the pattern of the movement in terms of time is based on the information gained by the aerial part of the stimulus.

Altogether these observations suggest a crosstalk between the roots and the aerial components of the plant. The higher velocities determined by the processing of the thin part of the stimulus need to be compensated by an earlier occurrence of key kinematical

parameters in order to establish a functional equilibrium between the roots and the shoot. To explain, the time of maximum tendrils velocity and aperture are crucial landmarks because they reflect the time at which the tendrils start to slow down and close upon the stimulus during the approaching phase, respectively. Uncertainty regarding that time might compromise the clasping of the pole. Therefore, if the information coming from the belowground part of the stimulus is inappropriate to maximize the chance of attachment, then modifications at the level of the aboveground organs of the plant are needed.

Turning to the comparison between the Thick-Below and the Control-Thin conditions no evidence for such crosstalk and no kinematical effects dictated by the perturbation occurred. It seems that what has been programmed on the basis of the below part of the stimulus (i.e., thick) well fits the requirements for grasping the above part of it (i.e., thin). Our preferred interpretation of these findings relies on the demonstration that for *P. sativum* plants (Chapters from 4 to 7) and climbing plants in general (Gianoli, 2015; Putz & Holbrook, 1992) grasping a thicker stimulus is much more demanding than grasping a thinner stimulus. Therefore, it might be easier to adapt a pattern of movement related to a thicker more demanding stimulus for grasping a less demanding thinner stimulus. In such circumstances, the effects of the perturbation are minimized and no difference with the control condition is found.

Altogether, these results indicate that the roots convey “*information*” to shoot that in turn can regulate growth and behavior accordingly. This is suggestive of a sort of functional equilibrium between the roots and shoot organs based on a signalling interactive process (Aiken & Smucker, 1996; Brouwer, 1963; Turner, 1986). It is well-known that these kinds of signals can determine an indirect root system effect on shoot attributes including leaf size and the capacity to orient leaves (Aiken & Smucker, 1996)

and, possibly, the dynamics of the tendrils for adapting to thickness (Chapters from 4 to 7).

At this stage, the natural question is: how do plants integrate the information from the above- and belowground organs in order to meet functional requirements? I am aware that present data say very little regarding possible physiological mechanisms underlying such effects, nevertheless some speculative thoughts can be advanced and discussed in the “General Discussion”.

CHAPTER 9



GENERAL DISCUSSION

9.1. The present research: an overview

The present thesis was driven by two main questions regarding climbing plants movement and behavior: are they able to perceive an element in the environment? And if so, are they able to code for the graspable properties of such element as to plan and execute an approach to grasp movement toward it, appropriately? In other words, are plants able to anticipate and respond depending on the varying states of the surrounding or they simply react passively to environmental elements? To answer these questions, I conducted a series of studies in which I used 3D kinematical analysis to characterize the approach-to-grasp movement of *P. sativum* plants in different environmental conditions.

The first experimental study (Chapter 4) was aimed to characterize, for the first time, the kinematical features of the approaching and grasping behavior in *P. sativum* plants and to assess if they were able to acknowledge the presence/absence of an element (i.e., stimulus) in the environment and to plan a movement toward it based on different structural properties. To this end, the approaching and grasping movement of *P. sativum* plants in an environment lacking a stimulus or toward a stimulus varying in thickness (i.e., thin or thick) and dimension (i.e., 2D or 3D) was assessed. Results showed that when the plants perceived the presence of the stimulus in the surrounding, they rapidly changed the direction of their circumnutating movement toward the stimulus as to approach and grasp it. When no stimulus was present, the plants circumnutate as to explore the environment searching for a potential stimulus and when they couldn't find it, they stopped moving and fell down. Then, results showed that *P. sativum* plants adjusted the kinematics of their

approaching and grasping movement depending on different stimulus thickness. To demonstrate that are the intrinsic properties of the stimulus, to determine the reported effects another investigation was conducted. The movement of *P. sativum* plants toward the ungraspable picture of either the thick or the thin stimulus used in the previous investigation, was assessed. Results pointed out that *P. sativum* plants were not able to acknowledge the presence of the ungraspable picture of the stimulus and to modulate the kinematics of their tendrils depending on what was represented in the photograph. Overall these results suggest that the plants are able to extract the “*graspable*” properties of the stimulus, elaborate them and produce suitable behavioral outputs in a similar manner as observed in different animal species (Castiello, 2005; Castiello & Dadda, 2019). *P. sativum* plants might be equipped with an efficient system for the processing and the evaluation of environmental information which allows them to adapt and implement a suitable motor response.

The next step of my work was driven by the curiosity to identify the possible rules that allow *P. sativum* plants to adjust the movement of their tendrils before the contact with the target stimulus, so to reduce the probability of possible errors. To assess this aspect, I conducted two experiments in which I investigated whether *P. sativum* plants can adjust the velocity of their tendrils (Chapter 5) and the node below the tendrils (Chapter 6) during their approach-to-grasp movement toward stimuli requiring a different level of accuracy. In the study reported in Chapter 5 I assessed if plants obey to the SAT phenomenon, which refers to the tendency for movement speed to co-vary with movement accuracy. In other words, if *P. sativum* plants can scale movement velocity as a function of the difficulty to coil a stimulus with different thickness. In the study reported in Chapter 6 I investigated if plants could correct online their action by means of secondary movements (i.e., submovements), and if their frequency production is influenced by the difficulty of

the task. Results from both studies suggest that *P. sativum* plants can reduce the probability of errors by using motor accuracy mechanisms akin of different animal species (Beamish, Bhatti, MacKenzie & Wu, 2006; Meyer et al., 1988). Overall the results for the first three experiments point out that even though plants are stuck in the ground, they are able to program and execute movements in a sophisticated manner for achieving their goals.

The second “*experimental*” part of my thesis was aimed to investigate the possible mechanism underlying thickness processing in plants. Specifically, I was driven by question of what is the contribution of the above (i.e., stem and tendrils) and the belowground organs of the plant in sensing and coding stimulus information. To this end, in my fourth study (Chapter 7) I assessed the contribution of the root system. The decision to focus my attention on the root system raised from the growing evidence reporting the role of the roots in a variety of behaviours in plants. For instance, it has been reported that the root system can perceive and detect the presence of physical objects in the belowground surrounding and modulate their growing behavior to avoid the contact with it (Falik et al., 2005; Semchenko et al., 2008; Wilson, 1967). Along these lines, I wondered whether the root system might be able to code for the intrinsic properties of the stimuli that they encounter in the soil. In Chapter 7 the movement of *P. sativum* plants toward a stimulus, which was either inserted in or lifted to the ground - so available (or not) to the root system – was assessed. Results showed that when the stimulus was detected by the root system, plants were able to modulate the kinematic of their tendrils depending on different stimulus thickness as previously observed (Chapters 4 and 5). When the information of the stimulus was not available by the roots because it was lifted to the ground, plants were not able to adjust the approaching and grasping movement of their tendrils with respect to different stimulus thickness. Overall these findings seem to suggest

a role for the root system in coding stimulus thickness. This conclusion, however, is puzzling and opens to the question of why does *P. sativum* plants rely on underground stimuli to drive aboveground behaviour? To better assess this aspect, in my fifth experiment (Chapter 8), the movement of *P. sativum* plants toward a stimulus with different thickness with respect to the below- and aboveground part of it was assessed. A group of plants was tested with a stimulus in which the belowground part was thin, and the aboveground part was thick, while another group of plants with a stimulus in which the belowground part was thick and the aboveground part was thin. Movements during perturbed trials were compared with one-sized stimuli that could be either thin or thick (i.e., control conditions). Results demonstrated the contribution of the root system in sensing, coding, and processing belowground information and how such information is evaluated and eventually modified at the level of the aerial part of the plant to fulfil the end-goal of the movement. The findings reported in Chapters 7 and 8 demonstrate the ability of roots to monitor and integrate numerous parameters simultaneously and to “translate” these sensory “experiences” into complex motoric responses (Gandar, 1983; Massa & Girloy, 2003a; Semchenko et al., 2008). But the results also pointed out the existence of a functional equilibrium reached through a crosstalk between the grounded and aerial components of the plant.

Despite the behavioural evidence provided above appears to be solid and consistent, what are the mechanisms underlying stimulus thickness coding could only be advanced at speculative level. In the next section some ideas regarding how this process might occur in plants will be put forward. Specifically, I shall focus on the sophisticated sensory mechanisms exhibited by plants as well on the crosstalk between the above- and belowground organs.

9.2. Sensory mechanisms

Several evidence suggest that plants are equipped with a wide range of sensory modalities (Karban, 2015). Here I shall consider some that may allow them to sense the surrounding and build a form of representation of it. These are a possible form of “*vision*” (Baluska & Mancuso, 2016; Crepy & Casal, 2015; Gavelis, Hayakawa, White, Gojobori, Suttle et al., 2015), acoustic perception (Gagliano et al., 2012), chemosensory perception (Ruynon et al., 2006; Weidenhamer, 2016) and electrical signalling (de Toledo, Parise, Simmi, Costa, Senko, Debono & Souza, 2019).

9.2.1. Aboveground possibilities

Vision

It has been advanced that plants have a sort of primitive visual system (Baluska & Mancuso, 2016; Crepy & Casal, 2015; Gavelis et al., 2015). In particular, the epidermical cells of the leaves might act as a primitive eye allowing the visual coding of the information of a stimulus in the environment (Baluska & Mancuso, 2016; Crepy & Casal, 2015; Gavelis et al., 2015). This idea is supported by evidence at both physiological and behavioural level.

At physiological level it has been demonstrated that single-celled eukaryotes algae (i.e., dinoflagellates) are characterized by an eye-like “*ocelloid*” consisting of subcellular analogues to a cornea, lens, iris, and retina which together are like to the camera-type eyes of some animals (Gavelis et al., 2015; Gehring, 2005). The light-sensitive signals in eyespots are rapidly converted into electrical and chemical signals to control flagella by means of the modification and the stacking of the thylakoid membranes originating from a chloroplast. This process, that may resemble a sort of vision, can be used by dinoflagellates to construct a very simple map of their local environment and to guide

assaults on other phytoplankton (i.e., photosynthesizing autotrophic organisms which are present in plankton; Gavelis et al., 2015).

At behavioural level it has been reported that higher plants such as the climbing wood vine *Boquilla trifoliolata* L. and the *A. thaliana* might present a specific “visual” system that allows them to sense and code the features (e.g., shape, color, ...) of different environmental stimuli (Baluska & Mancuso, 2016; Crepy & Casal, 2015; Gianoli & Carrasco-Urrà, 2014). For instance, the *B. trifoliolata* can adapt the shape, the color, and the size of its leaves according to the neighbouring plant in order to get protection from the attacks of herbivores. Importantly, the mimic of the leaves of the *B. trifoliolata* occurs even when the host plant is not in contact with it. This evidence suggests that the *B. trifoliolata* might be able to perceive the body shapes of neighboring plants through a sort of plant-specific vision. This ability might be also crucial to distinguish between plants within the same family (i.e., kin) and strangers. Recent findings have reported that photosensory receptors might allow the *A. thaliana* to recognize kin over stranger neighbors and to activate either cooperative or competitive behaviors (Crepy & Casal, 2015). The data included in my thesis cannot demonstrate that *P. sativum* plants use a sort of vision to extract the graspable properties of the stimulus in order to plan and execute an approach-to-grasp movement. However, the existence of a sort of primitive visual system in plants may be a likely candidate to explain how the graspable properties of the stimulus (e.g., thickness) could be perceived and processed online, that is before the contact with the stimulus, by *P. sativum* plants (Christie & Zurbriggen, 2020). Climbing plants, indeed, may use the information provided by the spatial distribution of light in their environment to recognise the shapes and forms of potential support in the environment, and to modulate the movement of their shoot and tendrils, accordingly.

Chemoreception

It has been hypothesized that plants might rely on the chemoreception of volatiles, which is a mechanism allowing for plant-environment interaction. Indeed, plants can emit and receive volatile blends and use these chemical cues to communicate with neighbors and localize a host plant (Karban, 2015; Parise et al., 2021; Ruynon et al., 2006). Then, it has been reported that some plants such as the *C. penatonata* use the emission and the perception of volatile compounds to localize potential hosts in the environment. For instance, Runyon and colleagues (2006) reported that the *C. penatonata* directs its growth toward both tomato plants (*Lycopersicon esculentum* L.) and the extracted tomato-plant volatiles. *C. penatonata* can also distinguish between different potential hosts [e.g., tomato vs impatiens (*Impatiens wallerana*) or wheat plants (*Triticum aestivum*)] and grow toward the preferred host (i.e., the tomato plant; Mescher, Ruynon & Consuelo, 2006; Ruynon et al., 2006). Further, it has been reported that the tendrils of climbing plants may function as a chemoreceptor system to detect the chemical cues of a contacted plant. For instance, the tendrils of the *C. japonica* plants tend to avoid coiling around a conspecific by means of the contact chemoreception for oxalate compounds, which are highly concentrated in *C. japonica* leaves (Fukano & Yamawo, 2015). The above evidence may explain how the tendrils of climbing or parasitic plants may sense different environmental elements and gain information from them by means of the reception and the processing of these volatile cues. These information might be used by plants to choose the most suitable support or to avoid the coiling around a conspecific. Here, *P. sativum* plants approached and grasped a neutral stimulus (i.e., wooden stick). Therefore, the chemoreception sensory mechanism seems not to be a likely candidate to explain how *P. sativum* plants sense and code the stimulus thickness information for the planning and the execution of a suitable motor command.

Proprioception

Plants morphology and architecture are constantly in flux given the presence of a wide range of internal (e.g., deformation of wall components, cell growth, ...) and external mechanical cues (e.g., fluttering leaves to stem flexion under tree weight and wind drag). Plants seem to be able to challenge these internal and external fluctuation by means of a form of proprioception. That is, a sensory mechanism involving the perception of cell- and tissue-shape changes linked to changes in body configuration, and the sense of tension and deformation of the plant's morphology and architecture (Moulia, Douady & Hamant, 2021; Sherrington, 1907).

Recent evidence has demonstrated the ability of plants to sense the relative position of below- and aboveground organs in the environment independently by external cues and orient their growth direction, properly (Bastien, Bohrd, Moulia & Douady, 2013; Hamant & Moulia, 2016; Okamoto, Ueda, Shimada, Tamura, Kato, Tasaka et al., 2015). However, given that the major movements in plants are linked to the growth, the study of plant proprioception is linked to experimentation on plant tropism. For instance, the gravitropism leads changing in the growth direction based on its angle to the gravity vector and it is a key determinant of the form and posture of plants (Bastien et al., 2013; Moulia, Bastien, Chauvet-Thiry & Leblanc-Fournie, 2019; Nakamura, Nishimura & Morita, 2019).

Proprioception in plants is mediated by the long actin filaments in elongating fiber cells, that, acting as a bending tensile sensor, perceive the plant's posture (Hamant & Moulia, 2016). These findings have been corroborated by studies on *A. thaliana* mutants defective in ACTIN-8, which exhibit peculiar reactions, such as hyperbending and sustained oscillations of the stem in response to gravity, tilting or other external perturbations (e.g., light stimuli; Okamoto et al., 2015). Proprioception, therefore, may

allow climbing plants to perceive the position of their tendrils and contribute to generating the necessary feedback information required for adjusting their aperture to the stimulus' thickness.

Electrical signalling

Plants might acquire information in the surrounding by means of electrical signaling activity. Several evidence has demonstrated that plants can create electrical fields, which might allow them to detect and code for the elements presented in the environment such as potential hosts (Bowker & Crenshaw, 2007a, b; Clarke, Morley & Robert, 2017; Clarke, Whitney, Sutton & Robert, 2013; Corbet et al., 1982; Parise et al., 2021). In this view, the tendrils of *P. sativum* plants might sense the thickness of the aboveground part of the stimulus by perceiving and processing the electric field arising from the charge carried by the interaction between the stimulus and the plant.

9.2.2. Belowground scenarios

Results of the experiments described in Chapters 7 and 8 suggest that the root system is involved in sensing the presence of a stimulus in the environment and its thickness. Further, it seems that the aerial components of the plants (i.e., stem and tendrils) are unable to code for the thickness of the stimulus on their own.

A growing number of studies have demonstrated the pivotal role of the root system in sensing, assessing, and responding to numerous signals (e.g., gravity, touch, humidity; Baluška et al., 2004; 2009; Hammond & White, 2011; Okada & Shimura, 1990; Trewavas, 2017; Wilson, 1967). For instance, roots stop developing downwards when they encounter a physical obstacle and instead begin to grow horizontally (Massa & Gilroy, 2003a). However, it is still unclear which signals are chiefly involved in the perception of a

stimulus and its features. In this view, three possible sensory mechanisms have been advanced.

Mechanical stimulation

Mechanical stimulation may provide to the plant stimulus thickness information via the interaction between the root cap and the stimulus. This idea is supported by evidence of Charles Darwin (1880) who observed that the roots of a wide range of plant species curve away from an obstacle in the soil after a touch stimulation. Since then, this phenomenon in the design of plant sensory experiments has been ignored. However, a study by Massa and Gilroy (2003), which was aimed to assess the interaction of touch and gravity in guiding roots downward through the soil, demonstrated that the roots of *A. thaliana* plants were able to grow vertically downward until the root tip encountered an horizontal glass barrier. When the root tip contacted the barrier the growth pattern of the root changed dramatically. The touch stimulation, which is provided by the contact between the roots and a glass barrier, lead the main roots to grow parallel to the obstacle while the tip of the roots maintain contact with the glass barrier. Upon reaching the edge of the barrier, the gravitropic response of the root once again became dominant and the root resumed normal downward growth. The touch stimulation, therefore, reduce the natural propensity to grow toward the gravity vector by promoting the growth far away from the perceived obstacles. Given that the cells of the root cap are the first to encounter obstacles in the soil, it has been suggested that they may be the site of gravity and touch sensing (Darwin & Darwin, 1880). In this view, the touch stimulation may provide to the whole plant body relevant information such as the position of the stimulus in the environment and its thickness. This idea is consistent with the fact that the results of the experiment reported in Chapter 7 showed that when the stimulus was lifted to the ground,

and thus not available by the roots, *P. sativum* plants were not able to localize the stimulus and adjust their approaching and grasping movement on the basis of different thickness.

Chemical signalling

Another possibility at the basis of stimulus thickness processing by the roots is concerned with the role of the root exudates, the cocktail of compounds secreted by roots, at the root cap, which allow plants to explore the soil environment and to gain information from it (Semchenko et al., 2008). Evidence suggests that plants can regulate their behaviour in response to abiotic and biotic factors not only by directly assessing resource availabilities, but also by employing complex chemical signalling. For example, root recognition and navigation around physical obstacles are in fact prevented when activated charcoal, which absorbs many compounds, is inserted into the substrate (Mahall & Callaway, 1992). Self-inhibition of root growth in the vicinity of obstructions, caused by the sensitivity of roots to localize accumulation of their own exudates, has been documented in highly artificial conditions, where obstructions were represented by nylon thread (Falik et al., 2005) and by pot walls (Semchenko et al., 2008). The sensitivity of roots to their own exudates accumulating in the vicinity of obstructions may ensure efficient root placement in obstructed substrate before resource uptake becomes directly affected by the aggregation of roots in limited space (Korenblum et al., 2020; Semchenko, Saar & Lepik, 2014). Exudate-mediated root-placement patterns alter the spatial and temporal distribution of root systems in the soil, ultimately altering root-soil interactions. In this view, the root system of the *P. sativum* plants may perceive the presence of the stimulus in the soil and gain information about its properties by means of the sensitivity of their roots to own exudates, which are produced close to the belowground part of the stimulus. That is, the

different thickness of the stimulus may influence the level of root exudates accumulating close to it leading the plant to perceive its thickness.

Acoustic perception

Plants might acquire information regarding the potential stimulus by emitting and responding to sounds. The ability of sound reception might have an important advantage for plants. Indeed, the belowground surrounding is characterized by a wide range of sounds which travel fast and carry different information such as the presence of predators or nutrient resources (i.e., water sources). For instance, it has been reported that the root of *P. sativum* plants use sounds to locate the water sources and direct their growing movement toward it (Gagliano, Grimonprez, Depczynski & Renton, 2017). Another example is provided by the *A. thaliana* which directs its root growth toward sounds with a frequency of 200 kHz, which is included in the spectrum of the sound of running water (Rodrigo-Moreno, Bazihizina, Azzarello, Masi, Tran, Bouteau, et al., 2017). Therefore, plants might be able to perceive environmental elements and to behave accordingly.

It has also been advanced that plants might be able to produce sounds by means of the broken branches under pressure or during the growing of the roots (Khait, Obolski, Yovel & Hadany, 2019; Tyree & Sperry, 1989). Further plants might acquire information about the surrounding by emitting sound and perceiving the returning echoes (Gagliano, 2013; Gagliano et al., 2012a, b; Mishra, Ghosh & Bae, 2016). In this view, climbing plants might use this behavioral strategy to detect a potential stimulus in the environment and to acquire information about its features such as thickness as to plan and execute a suitable approach-to-grasp movement toward it.

9.2.3. The crosstalk between the above- and belowground organs

Above the possibility that sensory mechanisms may explain how single organs may sense and code stimulus thickness information, the results outlined in Chapter 8 suggest a crosstalk between the below- and aboveground organs of the plants which allows to integrate different environmental signals as to optimize performance in highly dynamic environments. But how plants integrate the information from both the above- and belowground plant's organs in order to build a representation of the surrounding?

A possible explanation may rely on the propagation of electric signals between the root system and the shoot which may communicate and transmit signals to each other (Lüttge, 2021; van Bel, Furcj, Will, Buxa, Musetti & Hafke, 2014). For instance, the *D. muscipula* closes its trapping lobes to catch insects following the generation of electric phenomena which is provoked by the mechanical stimulation of its leave's hair by a prey (Burdon Sanderson, 1872). Another example is provided by the *M. pudica*, which close its leaves when they are touched by an external stimulus which is considered by the plant as dangerous. In this case, the closure of the leaves is linked with the transmission of the electrical signals among the whole body of the plant. In both examples, the transmission of the electrical signal from the touched region of the membrane of the plant to the neighboring ones is classified as an action potential (Volkov, Adesina & Jovanov, 2007). Further, it has been reported that the root system can generated spontaneously electrical signals which lead the creation of an electric field in the root rhizosphere (van West, Morris, Reid, Appiah, Osborne, Campbell et al., 2002). Thus, plants might use the propagation of the electrical signals to coordinate the activities and the physiological functions of the whole plant's organs to behave on their environment, properly (Fromm & Lautner, 2007). To date, four different types of transmission of electrical signals in plants are reported: (i) the wound potentials (Volkov & Brown, 2006); (ii) the action potentials

(AP; Hille, 1992); (iii) the slow wave potentials (SWP; Stahlberg & Cosgrove, 1992; 1996, 1997) and (iv) the systematic potentials (SP; Zimmerman et al., 2009; 2016). The first operates at a short distance, while the AP, the SWP and the SP play a fundamental role in the root-to-shoot signaling providing a two-way communication between roots and shoots. In sum, both electrical signals in the below- and aboveground organs of the plants may be propagated among the root-to-shoot (or *vice versa*) leading to the coordination of the activities and the physiological functions of the whole plant's organs which are fundamental to plan and execute a proper motor response toward the surrounding.

Another possibility is concerned with the propagation of chemical signals such as the growth hormones (i.e., auxin, cytokinin - CK, brassinosteroids - BRs, gibberellins - GA and strigolactones - SLs) which play a role in the development and growth of the new organs in the above- and belowground parts of the plant (Durbak, Yao & McSteen, 2012; Heil & Ton, 2008; Lüttge, 2021; Pieterse, van der Does, Zamioudis, Leon-Reyes & van Wees, 2012; Vanstraelen & Benkova, 2012; Torrey, 1976). Given that plants' body is characterized by multiple organs with different functions and nutritional requirements, both local and long-distance signaling are necessary for the coordination and the communication of nutrient status between each organ at the whole-plant level. In this view, it has been reported that the root-shoot and shoot-root translocation of hormones are given through the xylem (i.e., the plant vascular tissue that conveys water and dissolved minerals from the roots to the rest of the plant and provides physical support) and the phloem (i.e., the plant tissue that conducts sugars from the leaves to the other parts of the plant) respectively (Notaguchi & Okamoto, 2015; Symons & Reid, 2004). For instance, the cytokinins like trans-zeatin synthesized in the root have been detected in the xylem sap and they play a role in the shoot growth (Takei, Sakakibara, Taniguchi & Sugiyama, 2001; Takei, Ueda, Aoki, Kuromori, Hirayama, Shinozaki et al., 2004). Then, the shoot controls

the export of cytokinin from the root to the shoot and this is dependent on the feedback signal generated from the branching regulator RMS4 (RAMOSUS4) acting in the shoot in *P. sativum* plants (Beveridge, Murfet, Kerhoas, Sotta, Miginiac & Rameau, 1997). In addition, cytokinin export to the shoot is sensitive to nitrogen, which lead to changes in root and shoot architecture (e.g., leaf expansion; Alvarez, Vidal & Gutierrez, 2012; Dodd, Ngo, Turnbull & Beveridge, 2004; Forde, 2002). Both miRNAs (Vidal, Araus, Lu, Parry, Green, Coruzzi & Gutiérrez, 2010) and auxins are possible candidates for the shoot-to-root signals mediating systemic nitrogen responses. For example, the nitrate supply to roots is linked to lateral root responses through the modulation of shoot-to-root auxin transport in *A. thaliana* (Guo, Chen, Zhang & Mi, 2005). The driving and transmission processes underlying the growth and motor behavior in plants occur through changes in hormone gradients across cells. This mechanism is also concerned with the correct speed and direction of the movement to be generated (e.g., active bending of the organs, reconfiguration of the shape of the plant's organs, ...; Moulia et al., 2021). In this view, the feedback received by the sensing and posture regulation of the tendrils and/or the root system of the plants may allow the acquisition of the information regarding stimulus properties. In turn such information might be transmitted to the whole body of the plant to generate suitable motor commands.

9.3. Linking action to cognition

It is now time to re-take the theories and the concepts outlined in Chapter 1. There, an action is defined as intentional or “*goal-directed*” when it is tuned to the task and its execution is under voluntary control of the agent. And, the main goal of the action persists in the agent's phenomenological experience throughout the time the action is unfolding and until it has been completed. Here, *P. sativum* plants seem to be able to actively explore

the surrounding, anticipate what is going to happen in it, “*make a decision*” freely based on the information provided from the surrounding and regulate their behavior and actions accordingly to enhance their chances to satisfy their endogenous needs (e.g., reach the light source). The present results also suggest that the behavior of *P. sativum* plants seems not to be a merely collection of an automatic stimulus-response ensemble but a globally organized cohering unit. In this view, plants could be defined as “*cognitive*” agents in the general sense used by the post-cognitivism theories (i.e., enactivism, embodied and extended cognition; see Chapter 1).

Overall the results outlined in my thesis suggest that *P. sativum* plants might be able to have a truthful metabolic representation of the external world. In fact: (i) climbing plants move and choose a support on the basis of their endogenous need to seek the sun. An unsuitable or no support fails to satisfy their intention; (ii) climbing plants control their circumnutation movement and interact with their surroundings without any externally imposed compulsions; it is a behavior that arises from their very nature; (iii) climbing plants act freely and are able to terminate the process when the support is not suitable or when there is no support. This suggests that the presence of a brain or a nervous system would not always appear to be necessary for the expression of cognitive abilities. But how can plants do that without a brain? According to the classical cognitive view, cognition arises only when the system is able to exploit mental representations. That is, mental states that are subjected to semantic evaluation (i.e., true or false, accurate or inaccurate, ...) and take the form of propositional attitudes (e.g., beliefs, thoughts, ...), which are not present in brainless organisms. In this view, it is evident that it would be impossible for plants to show cognitive capabilities. However, there is no reason to suppose that cognition only depends on semantically evaluable metabolic representations. Furthermore, according to the new post-cognitivism theories (i.e., enactivism, embodied and extended cognition) a

system is defined as cognitive when it is autonomous, open to the exploration of its environment in order to meet its own needs and goals - instead of simply reacting to the external cues - and it is capable of actively regulating its sensorimotor coupling in context-sensitive ways. Further, the ecological-enactive approach claims that complex cognitive capacities such as the ones that involve representations are deeply rooted in the more basic processes that enable biological organisms to survive and maintain their integrity in a dynamical environment. In this view, cognition does not necessarily depend on mental representations, and it could be extended from single cell organisms to human beings, including plants. Further, it has been advanced that cognitive activities depend on the structural supports or resources that the environment offers. That is, what an organism can do with the perceived object in its surrounding (i.e., affordances; Gibson, 1979). However, given that the same environment can provide various affordances to different organisms or to the same organism at different times, the key to understand cognition resides on the relation between the environment and the current intentions and capabilities of the organism. In this view, the abilities of climbing plants to actively explore the environment searching for a potential support and select the most appropriate one to reach the lightest exposure exemplifies how the concept of affordances can be applied also to brainless organism such as plant, which, in turn, may be considered as cognitive agents.

Having said that the use of terms as “*cognitive*”, “*intention*”, “*representation*” referring to brainless organism could arise some concerns. But do plants lack of a brain? Given that plants are sessile organism in nature, they are not characterized by a centralized system in which the environmental cues perceived by plants’ tissues are processed and encoded before being transmitted to the other parts of the plant’s body (Trewavas 2016). However, in the last paragraph of the book “*The power of movement in plants*” by Charles and Francis Darwin (1880) it has been advanced that the root system of the plants may be

considered as a brain-like organ (though the entire plant can be considered as a brain), resembling that observed in lower animals. Indeed, the tip of the radicle is able to perceive and process a wide range of belowground cues and to translate these sensory experiences into complex motor behavior which are expressed by the adjoining part of the plant (Darwin & Darwin, 1880). This idea, which is named “*the root-brain hypothesis*” has been taken over by recent evidence demonstrating that the growing root apices represent sensory areas in which environmental signals are perceived, processed, and then transmitted to the whole-body allowing the execution of adaptive behaviors (Baluška, 2010; Baluška et al., 2004; 2006; 2009; 2010). In this view, each root apex may be considered as a brain-like units of the nervous system of plants, which are interconnected via vascular strands with their polarly-transported auxin (Baluška et al., 2006). Then, the modular bioelectrical activities of the interconnected cells in plants (*see* paragraph 9.2.3.) may be considered as a dynamic proto-neural networks which may ensure and support the constant informational, learning, and cognitive processing (Baluška, 2010; Baluška & Mancuso, 2013; 2016; Baluška & Levin, 2016; Debono, 2013; de Loof, 2016; de Toledo et al., 2019; Trewavas 2016). Indeed, the signals locally processed (e.g., nutrients, drought stress, ...) should be integrated and transmitted at the plant level by means of local and long-distance signaling (e.g., action potentials) which lead the coordination of the physiological functions of the whole plant’s organs to behave on the environment, properly (Baluška & Mancuso 2013; Brenner, Stahlberg, Mancuso, Vivanco, Baluška & van Volkenburgh, 2006; Fromm & Lautner, 2007; Jones & Dangl, 2006; Huber & Bauerle, 2016; Stahlberg, 2006). For instance, it has been reported that the induction of an action potential in the roots of the *Z. mays* plant is rapidly transmitted to the leaves through the phloem affecting the rate of the carbon dioxide (CO²) assimilation at the leaves level (Fromm & Fei, 1998).

In light of the above it might make sense to think at the roots as organs within which environmental cues are perceived and processed (Baluška et al., 2004; 2009; Castiello, 2020; Parise et al., 2020; Segundo-Ortin & Calvo, 2019; Trewavas, 2009; 2016; 2017). Further, other evidence suggests the existence of a dynamic proto-neural networks based on electrical signaling activities, which allows the transmission of the sensory information at the whole-body of the plants leading to adaptive and flexible behavior.

9.4. Some considerations on the similarities between animals and plants

To sum up, results of my experiments have demonstrated that *P. sativum* plants are able to perceive an element in the environment and to plan a movement based on the thickness of a stimulus. In other words, the present thesis has demonstrated that *P. sativum* plants are able to act “*intentionally*” on their surroundings, in a similar manner as observed in animal species including human beings.

In my work the targeted movement is grasping. Humans and non-human animals (e.g., monkeys, tetrapods, ...) have evolved prehensile abilities which are shaped by both the object features (e.g., size, dimension, ...) and the overarching goal of the action (for reviews see Castiello, 2005; Castiello & Dadda, 2019). The reach-to-grasp movement is defined as a first a progressive opening of the grip, followed by a gradual closure until the aperture of the fingers matches the structure of the object. When considering size, the object property targeted in the present series of studies, kinematic signatures are evident. Movement time is prolonged, the velocity profile is higher, the deceleration time (i.e., from peak velocity to the end of the movement) is shorter, and the maximum grip aperture is wider and delayed for larger compared to smaller objects (Fig. 9.1. a; Castiello, 2005; Castiello & Dadda, 2019; Jeannerod, 1981).

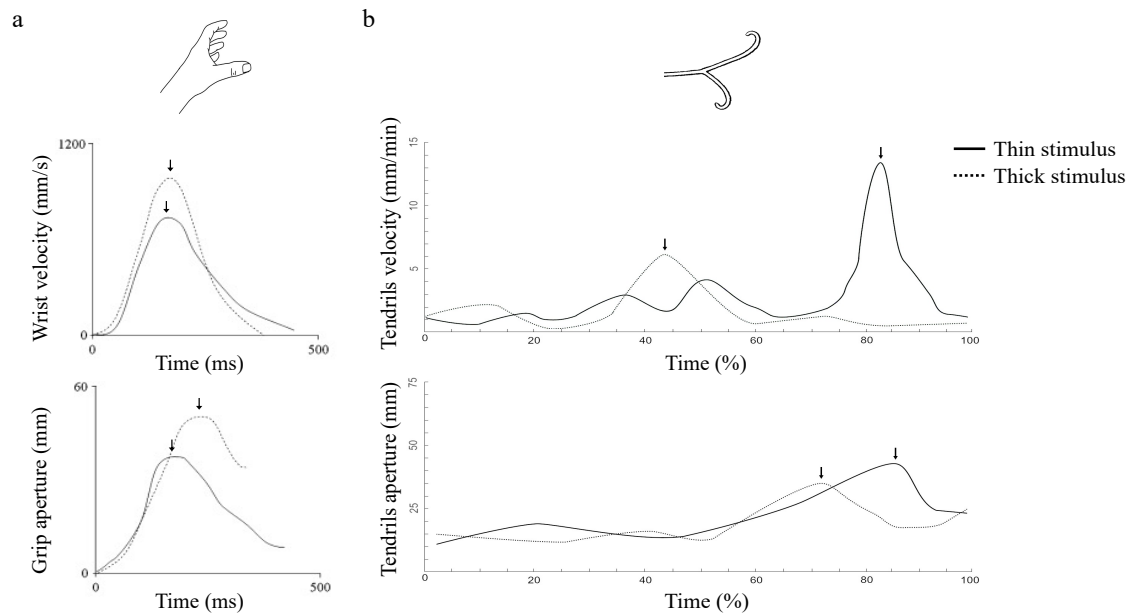


Fig. 9.1. (a) The time plots of wrist velocity and grip aperture for a representative hand movement directed to either a thin or a thick stimulus. As highlighted by means of the black arrows the peak arm velocity and the maximum aperture of the grip occurred later for the larger than for the smaller stimulus. (from Castiello & Dadda, 2019). (b) The time plots of tendril velocity and tendril aperture profiles for movements performed towards either a thick or a thin stimulus. As highlighted by means of the black arrows the peak velocity and the maximum aperture of the tendril occurred later for the thinner than the thicker stimulus.

In terms of SAT humans and non-humans animals produce slower movements when the task requires reaching and grasping a smaller than a larger target. Slower movements, indeed, allow the acquisition of more information on the target by means of proprioceptive and visual feedback, which information are used to adjust any spatial discrepancy between hand and target position implementing corrective adjustments (Meyer et al., 1988; Plamondon & Alimi, 1997). Then, given that human beings experience more difficulty to interact with a smaller than a larger target (Fitts, 1954) they produce more corrective adjustments (i.e., secondary submovements) during the approaching and grasping movement toward small stimuli (Fradet et al., 2008). In other words, when the task requires more precision, more secondary submovements are needed to reduce the end-point variability of an effector (e.g., arm for humans), and thus, the probability that the effector falls outside of the target bound. Therefore, in different animal

species the frequency of submovements is larger when the task requires to interact with smaller stimuli than larger ones.

With this in mind, the pattern of the plants' approaching and grasping movement it may appear exactly the opposite of that showed by animals (Fig. 9.1. b). Though, as I shall explain, this might not be necessarily the case. My results showed that the approaching and grasping movement of *P. sativum* plants is faster and the tendrils' aperture is wider for thinner over thicker stimulus. And, the time at which the maximum tendrils velocity and aperture occurred is earlier for thicker than thinner stimuli (Chapters 4, 5, 7 and 8). Further plants exhibit more difficulty during the approaching and grasping of a thicker than a thinner stimulus and they tend to produce more secondary submovements when they must clasp a stimulus with a greater diameter (Chapters 5 and 6).

A possible explanation may reside in the fact that for climbing plants grasping a thicker stimulus is a more energy consuming process than grasping a thinner one. Indeed, plants may increase the tendrils length to efficiently coil the stimulus (Rowe et al., 2006) and to strengthen the tensional forces to resist gravity (Gianoli, 2015) consuming higher adenosine triphosphate (ATP; Putz & Holbrook, 1992). Therefore, the reduction of the movement velocity and the containment of the aperture of the tendrils may help plants to save energy for the grasping phase and reduce the risk of errors assuring a firm attachment to the stimulus. Moreover, the reduction of movement velocity may lead plants to lengthen the time window within which tendrils establish contact points upon the stimulus. This process may be useful for plants to accumulate more information about the stimulus's physical characteristics and to implement corrective adjustments of the tendrils trajectories to select more accurately the contact point to the stimulus and to clasp it more firmly (Putz & Holbrook, 1992).

Overall animals and plants adopt the same basic principles for interacting with stimuli in the environment. As animals, plants seem to be structured by sensory mechanisms, which allow the detection and the processing of the information about the properties of stimulus. Plants seem to be able to coordinate their physiological activities by means of the transmission electrical signaling through the whole-body to produce adaptive behaviours and motor commands without the need of a CNS. Collectively, the present observations raise questions at an evolutionary level about the ubiquity of this mechanism in other species. Despite plants and animals are two unique evolutionary adaptations for multicellular life, each depending on unique kingdom-specific sets of cells, tissues and organs, plants might have evolved a more specialized and differentiated signaling networks and mechanisms based on a common toolset from our unicellular common ancestor.

9.5. Conclusive remarks

There were some questions that “*walked with me*” during my Ph.D. and the drafting of the present thesis. Questions that are partially answered in my thesis and that I hope will find a response in my future work. From a theoretical perspective can plants be defined as cognitive organisms? My work has demonstrated that *P. sativum* plants are able to use the information of the environment to guide and control their motor behavior in an intentional manner. That is, *P. sativum* plants seem to be able to perceive and integrate environmental information to achieve their goals rather than simply reacting to the external impingements. The idea that the root tip functions as a “*command center*” (Darwin & Darwin, 1880; Baluška et al., 2004) or, as recently pointed out, as a “*cognitive center*” (Baluška et al., 2009; Castiello, 2020; Hodge, 2004; 2009; Hodge et al., 2009; Parise et al., 2020; Segundo-Ortin & Calvo, 2019; Trewavas, 2009; 2016; 2017) is

appealing and one that needs to be pursued. In this respect I envisage a series of studies looking at the kinematics of roots together with the analysis of roots exudates. I foresee that this body of data will provide invaluable instruments to theorize about possible alternative cognitive architectures.

In terms of sensory systems my results suggest a possible crosstalk between the above- and the belowground part of the plants. In this view, the adoption of different sensory systems (i.e., primitive visual system and/or the electrical signaling activity; Baluska & Mancuso, 2016; Clarke et al., 2013; 2017; Crepy & Casal, 2015) might help plants to monitor and process different aerial cues. Then, the integration of the information from both the above- and belowground plant's organs might provide to the plant a full reconstruction of the surrounding leading the flexibly adaptation of their behavior to different environmental situations. This taps into the construction of representation from sensory information that may lead the regulation of sensorimotor coupling in context-sensitive ways. I would like to tackle this issue by using machine learning techniques to understand how such representations are built.

I am well aware that studies in a natural setting are of course warranted to verify the relevance of these findings in an ecological context (Calvo & Trewavas, 2020). The fact that a plant responds in a particular way in an unnatural environment does not necessarily mean it will do so in the natural one. Integrating these results with ecological observations may lead to identifying specific behavioral cues during stimulus sensing processes in plants, enhancing our knowledge about plant behavior. Nevertheless, present findings suggest that root signals to shoots can adapt the plant growth processes such as the growth and the behaviour of tendrils. Knowledge of root signals regulating whole plant growth processes suggests new analytical and experimental tools for integrated analysis of plant phasic development, optimal growth, and ecological fitness. Analytical

investigations provide the evidence required to discern direct and indirect effects of root function on whole plant growth.

Future studies aimed at investigating both the kinematic profiles of the root and shoot growth in natural and/or non-natural settings are needed to further explore the functional equilibrium and interactivity between the above- and below plants' organs. The integrated analysis of whole plant growth responses at kinematical level might, indeed, might provide a new suite of investigative tools that may expand our understanding of rhizosphere dynamics.

REFERENCES



- Aiken, R. M. & Smucker, A. J. M. (1996). Root system regulation of whole plant growth. *Annual Review of Phytopathology*, 34(1), 325-346.
Doi:10.1146/annurev.phyto.34.1.325
- Alvarez, J. M., Vidal, E. A., & Gutierrez, R. A. (2012). Integration of local and systemic signaling pathways for plant N responses. *Current Opinion in Plant Biology*, 15(1), 185–191. Doi:10.1016/j.pbi.2012.03.009
- Arnal, C. (1953). Recherches sur la nutation des coleoptiles. II. Nutation et regeneration physiologique. *Annales Universitatis Saraviensis*, 2, 186-203.
- Atamian, H. S., Creux, N. M., Brown, E. A., Garner, A. G., Blackman, B. K., & Harmer, S. L. (2016). Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits. *Science*, 353(6299), 587-590. Doi:10.1126/science.aaf9793
- Baillaud, L. (1962). Les mouvements d’exploration et d’enroulement des plantes volubiles. *Handbuch der Pflanzenphysiologie*, 17, 635 –715.
- Bais, H. P., Park, S. W., Weir, T. L., Callaway, R. M., & Vivanco, J. M. (2004). How plants communicate using the underground information superhighway. *Trends in Plant Science*, 9(1), 26-32. Doi:10.1016/j.tplants.2003.11.008
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, 57, 233-266.
Doi:10.1146/annurev.arplant.57.032905.105159
- Baldwin, I. T., Halitschke, R., Paschold, A., Von Dahl, C. C., & Preston, C. A. (2006). Volatile signaling in plant-plant interactions: “talking trees” in the genomics era. *Science*, 311(5762), 812-815. Doi:10.1126/science.1118446

- Baluška, F. (2010). Recent surprising similarities between plant cells and neurons. *Plant Signaling & Behavior*, 5(2), 87-89. Doi:10.4161/psb.5.2.11237
- Baluška, F., & Levin, M. (2016). On having no head: cognition throughout biological systems. *Frontiers in Psychology*, 7, 902. Doi:10.3389/fpsyg.2016.00902
- Baluška, F., & Mancuso, S. (2013). Root apex transition zone as oscillatory zone. *Frontiers in Plant Science*, 4, 354. Doi:10.3389/fpls.2013.00354
- Baluška, F., & Mancuso, S. (2016). Vision in plants via plant-specific ocelli? *Trends in Plant Science*, 21(9), 727–730. Doi:10.1016/j.tplants.2016.07.008
- Baluška, F., Barlow, P. W., Volkmann, D., & Mancuso, S. (2007). *Gravity-related paradoxes in plants: Plant neurobiology provides the means for their resolution*. In Witzany, G. (Ed.), *Biosemiotics in transdisciplinary context: Proceedings of the Gathering in Biosemiotics*. (pp. 9–35).
- Baluška, F., Lev-Yadun, S., & Mancuso, S. (2010). Swarm intelligence in plant roots. *Trends in Ecology and Evolution*, 25(12), 682–683.
Doi:10.1016/j.tree.2010.09.003
- Baluška, F., Mancuso, S., Volkmann, D., & Barlow, P. (2004). Root apices as plant command centres: The unique ‘brain-like’ status of the root apex transition zone. *Biologia*, 59(13), 7–19.
- Baluška, F., Mancuso, S., Volkmann, D., & Barlow, P. W. (2009). The ‘root-brain’ hypothesis of Charles and Francis Darwin: Revival after more than 125 years. *Plant Signaling and Behavior*, 4(12), 1121–1127. Doi: 10.4161/psb.4.12.10574
- Baluška, F., Mancuso, S., Volkmann, D., & Barlow, P. W. (2010). Root apex transition zone: a signalling–response nexus in the root. *Trends in Plant Science*, 15(7), 402-408. Doi:10.1016/j.tplants.2010.04.007

- Barlow, P. W. (2006). *Charles Darwin and the plant root apex: Closing a gap in living systems theory as applied to plants*. In Baluška, F., Mancuso, S., & Volkmann D. (Eds.), *Communication in plants*. (pp. 37–51). Springer. Doi:10.1007/978-3-540-28516-8_3
- Barlow, P. W. (2010). Plant roots: Autopoietic and cognitive constructions. *Plant Root*, 4, 40–52. Doi:10.3117/plantroot.4.40
- Bastien, R., & Meroz, Y. (2016). The kinematics of plant nutation reveals a simple relation between curvature and the orientation of differential growth. *PLoS Computational Biology*, 12(12), e1005238. Doi:10.1371/journal.pcbi.1005238
- Bastien, R., Bohr, T., Moulia, B., & Douady, S. (2013). Unifying model of shoot gravitropism reveals proprioception as a central feature of posture control in plants. *Proceedings of the National Academy of Sciences*, 110(2), 755-76. Doi:10.1073/pnas.1214301109
- Basu, P., Pal, A., Lynch, J. P., & Brown, K. M. (2007). A novel image-analysis technique for kinematic study of growth and curvature. *Plant physiology*, 145(2), 305-316. Doi:10.1104/pp.107.103226
- Bateson, G. (1985). *Mind and Nature. A Necessary Unity*. Fontana, London, UK
- Batiza, A. F., Schulz, T., & Masson, P. H. (1996). Yeast respond to hypotonic shock with a calcium pulse. *Journal of Biological Chemistry*, 271(38), 23357-23362. Doi:10.1074/jbc.271.38.23357
- Bauer, G., Klein, M. -C., Gorb, S. N., Speck, T., Voigt, D., & Gallenmüller, F. (2011). Always on the bright side: The climbing mechanism of Galium aparine. *Proceeding of the Royal Society Biological Sciences*, 278(1715), 2233–2239. Doi:10.1098/rspb.2010.2038

- Bazzaz, F. A. (1991). Habitat selection in plants. *The American Naturalist*, *137*, S116-S130. Doi:10.1086/285142
- Beamish, D., Bhatti, S. A., MacKenzie, I. S., & Wu, J. (2006). Fifty years later: a neurodynamic explanation of Fitts' law. *Journal of The Royal Society Interface*, *3*(10), 649-654. Doi:10.1098/rsif.2006.0123
- Becchio, C., Sartori, L., Bulgheroni, M., & Castiello, U. (2008). Both your intention and mine are reflected in the kinematics of my reach-to-grasp movement. *Cognition*, *106*(2), 894-912. Doi:10.1016/j.cognition.2007.05.004
- Beggs, W. D. A., & Howarth, C. I. (1972). The movement of the hand towards a target. *Quarterly Journal of Experimental Psychology*, *24*(4), 448–453. Doi:10.1080/14640747208400304
- Beveridge, C. A., Murfet, I. C., Kerhoas, L., Sotta, B., Miginiac, E. & Rameau, C. (1997). The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant rms4. *The Plant Journal*, *11*, 339–345. Doi:10.1046/j.1365-313X.1997.11020339.x
- Bickhard, M. (2008). *Is embodiment necessary?* In: Calvo, P. & Gomila, T.(Eds.), *Handbook of Cognitive Science: An Embodied Approach* (pp. 29-40). Elsevier.
- Bonato, B., Peressotti, F., Guerra, S., Wang, Q. & Castiello, U. (2021). Cracking the code: a comparative approach to plant communication. *Communicative & Integrative Biology*, *14*(1), 176-185. Doi:10.1080/19420889.2021.1956719
- Bowker, G. E., & Crenshaw, H. C. (2007a). Electrostatic forces in wind-pollination—Part 1: Measurement of the electrostatic charge on pollen. *Atmospheric Environment*, *41*(8), 1587-1595. Doi:10.1016/j.atmosenv.2006.10.047

- Bowker, G. E., & Crenshaw, H. C. (2007b). Electrostatic forces in wind-pollination—Part 2: simulations of pollen capture. *Atmospheric environment*, *41*(8), 1596-1603.
Doi:10.1016/j.atmosenv.2006.10.048
- Braam, J. (2005). In touch: Plant responses to mechanical stimuli. *The New Phytologist*, *165*(2), 373–389. Doi:10.1111/j.1469-8137.2004.01263.x
- Brenner, E. D., Stahlberg, R., Mancuso, S., Vivanco, J., Baluška, F., & van Volkenburgh, E. (2006). Plant neurobiology: an integrated view of plant signaling. *Trends in plant science*, *11*(8), 413-419. Doi:10.1016/j.tplants.2006.06.009
- Britz, S. J. & Galston, A. W. (1982). Physiology of movements in stems of seedling *Pisum sativum* L. cv Alaska. *Plant Physiology*, *70*(1), 1401-4. Doi:10.1104/pp.70.1.264
- Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and environmental microbiology*, *74*(3), 738-744. Doi:10.1128/AEM.02188-07
- Brouwer, R. (1963). Some aspects of the equilibrium between overground and underground plant parts. *Jaarboek van het Instituut voor Biologisch en Scheikundig onderzoek aan Landbouwgewassen, 1963*, 31-39.
- Brown, A. H. (1993). Circumnutations: from Darwin to space flights. *Plant Physiology*, *101*(2), 345–348. Doi:10.1104/pp.101.2.345
- Brown, A. H., Chapman, D. K., Lewis, R. F., & Venditti, A. L. (1990). Circumnutations of sunflower hypocotyls in satellite orbit. *Plant Physiology*, *94*(1), 233-238.
Doi:10.1104/pp.94.1.233
- Burdon Sanderson, J. (1872). Note on the electrical phenomena which accompany stimulation of the leaf of *Dionaea muscipula* Ellis. *Philosophical Proceedings of the Royal Society London*, *21*(139-147), 495–496. Doi:10.1098/rspl.1872.0092

- Burdon-Sanderson, J. S. (1873). I. Note on the electrical phenomena which accompany irritation of the leaf of *Dionæa muscipula*. *Proceedings of the Royal Society of London*, 21(139-147), 495-496. Doi:10.1098/rspl.1872.0092
- Burns, J. G., & Rodd, F. H. (2008). Hastiness, brain size and predation regime affect the performance of wild guppies in a spatial memory task. *Animal Behaviour*, 76(3), 911–922. Doi:10.1016/j.anbehav.2008.02.017
- Cahill, J. F., & McNickle, G. G. (2011). The behavioral ecology of nutrient foraging by plants. *Annual Review of Ecology, Evolution, and Systematics*, 42(1), 289–311
Doi:10.1146/annurev-ecolsys-102710-145006
- Cahill, J. F., McNickle, G. G., Haag, J. J., Lamb, E. G., Nyanumba, S. M., & Clair, C. C. S. (2010). Plants integrate information about nutrients and neighbors. *Science*, 328(5986), 1657-1657. Doi:10.1126/science.1189736
- Calaghan, S. C., & White, E. (1999). The role of calcium in the response of cardiac muscle to stretch. *Progress in Biophysics and Molecular Biology*, 71(1), 59-90.
Doi:10.1016/S0079-6107(98)00037-6
- Calvo, P. (2007). The quest for cognition in plant neurobiology. *Plant Signaling & Behavior*, 2(4), 208–211. Doi:10.4161/psb.2.4.4470
- Calvo, P. (2017). What is it like to be a plant?. *Journal of Consciousness Studies*, 24(9-10), 205-227.
- Calvo, P., & Friston, K. (2017). Predicting green: Really radical (plant) predictive processing. *Journal of the Royal Society Interface*, 14(131), 20170096.
Doi:10.1098/rsif.2017.0096
- Calvo, P., & Keijzer, F. A. (2009). *Cognition in plants*. In Baluška, F. (Ed.), *Plant–environment interactions: Behavioral perspective* (pp. 247–266). Springer-Verlag.

- Calvo, P., & Trewavas, A. (2021). Cognition and intelligence of green plants. Information for animal scientists. *Biochemical and Biophysical Research Communications*, 564, 78–85. Doi:10.1016/j.bbrc .2020.07.139
- Calvo, P., Gagliano, M., Souza, G. M., & Trewavas, A. (2020). Plants are intelligent, here's how. *Annals of Botany*, 125(1), 11-28. Doi:10.1093/aob/mcz155
- Calvo, P., Raja, V., & Lee, D. N. (2017). Guidance of circumnutation of climbing bean stems: An ecological exploration. *BioRxiv*, 122358. Doi: 10.1101/122358
- Caraco, T., Martindale, S., & Whittam, T. S. (1980). An empirical demonstration of risk-sensitive foraging preferences. *Animal Behaviour*, 28(3), 820-830.
Doi:10.1016/S0003-3472(80)80142-4
- Carrasco-Urra, F., & Gianoli, E. (2009). Abundance of climbing plants in a southern temperate rain forest: Host tree characteristics or light availability? *Journal of Vegetation Science*, 20(6), 1155–1162. Doi:10.1111/j.1654-1103.2009.01115.x
- Carsten, L. D., Juola, F. A., Male, T. D. & Cherry, S. (2002). Host associations of lianas in a south-east Queensland rain forest. *Journal of Tropical Ecology*, 18(1), 107 –120.
Doi:10.1017/S0266467402002067
- Castiello, U. (2005). The neuroscience of grasping. *Nature Reviews Neuroscience*, 6(9), 726-736. Doi:10.1038/nrn1744
- Castiello, U. (2020). (Re) claiming plants in comparative psychology. *Journal of Comparative Psychology*, 135(1), 127. Doi:10.1037/com0000239
- Castiello, U., & Guerra, S. (2021). Psicologia vegetale: alle radici della cognizione. *Giornale italiano di psicologia*, 2020(3-4), 683-708. Doi:10.1421/100053
- Castiello, U., & Dadda, M. (2019). A review and consideration on the kinematics of reach-to-grasp movements in macaque monkeys. *Journal of Neurophysiology*, 121(1), 188-204. Doi:10.1152/jn.00598.2018

- Cazalis, R., Carletti, T. & Cottam, R. (2017). The living organism: strengthening the basis. *BioSystems*, 158, 10–16. Doi:10.1016/j.biosystems.2017.04.007.
- Ceccarini, F., & Castiello, U. (2018). The grasping side of post-error slowing. *Cognition*, 179, 1-13. Doi:10.1016/j.cognition.2018.05.026
- Chemero, A. (2013). Radical embodied cognitive science. *Review of General Psychology*, 17(2), 145-150. Doi:10.1037/a0032923
- Chittka, L., Skorupski, P., & Raine, N. E. (2009). Speed–accuracy tradeoffs in animal decision making. *Trends in Ecology & Evolution*, 24(7), 400–407. Doi:10.1016/j.tree.2009.02.010
- Ciesielski, T. (1872). Untersuchungen u“ber die Abwärtskrümmung der Wurzel. *Beitr Biol Pflanz*, 1, 1–30.
- Clark, A. (2008). *Supersizing the mind: Embodiment, action, and cognitive extension*. OUP USA.
- Clark, A. (2013). Whatever next? Predictive brains, situated agents, and the future of cognitive science. *Behavioral and Brain Sciences*, 36(3), 181-204. Doi:10.1017/S0140525X12000477
- Clark, A., & Chalmers, D. (1998). The extended mind. *Analysis*, 58(1), 7–19.
- Clark, L. J., Whalley, W. R., & Barraclough, P. B. (2003). *How do roots penetrate strong soil?*. In *Roots: The Dynamic Interface Between Plants and the Earth* (pp. 93-104).
- Clark, R. T., MacCurdy, R. B., Jung, J. K., Shaff, J. E., McCouch, S. R., Aneshansley, D. J., & Kochian, L. V. (2011). Three-dimensional root phenotyping with a novel imaging and software platform. *Plant Physiology*, 156(2), 455-465. Doi:10.1104/pp.110.169102

- Clarke, D., Morley, E., & Robert, D. (2017). The bee, the flower, and the electric field: electric ecology and aerial electroreception. *Journal of Comparative Physiology A*, 203(9), 737-748. Doi:10.1007/s00359-017-1176-6
- Clarke, D., Whitney, H., Sutton, G., & Robert, D. (2013). Detection and learning of floral electric fields by bumblebees. *Science*, 340(6128), 66-69.
Doi:10.1126/science.1230883
- Corbet, S. A., Beament, J., & Eisikowitch, D. (1982). Are electrostatic forces involved in pollen transfer? *Plant, Cell & Environment*, 5, 125–129. Doi:10.1111/1365-3040.ep11571488
- Costa, M., Goldberger, A. L., & Peng, C. K. (2005). Multiscale entropy analysis of biological signals. *Physical Review E*, 71(2), 021906.
Doi:10.1103/PhysRevE.71.021906
- Craver, C. (2007). *Explaining the brain: What a science of the mind-brain could be*. Oxford University Press.
- Crepy, M. A., & Casal, J. J. (2015). Photoreceptor-mediated kin recognition in plants. *New Phytologist*, 205(1), 329-338. Doi:10.1111/nph.13040
- Crick, F. (1996). The astonishing hypothesis: the scientific search for the soul. *The Journal of Nervous and Mental Disease*, 184(6), 384.
- Crick, F., & Clark, J. (1994). The astonishing hypothesis. *Journal of Consciousness Studies*, 1(1), 10-16.
- Christie, J. M., & Zurbruggen, M. D. (2021). Optogenetics in plants. *New Phytologist*, 229(6), 3108-3115. Doi: 10.1111/nph.17008
- Croteau, E. K. (2010). Causes and Consequences of Dispersal in Plants and Animals. *Nature Education Knowledge*, 3, 12.
- Darwin, C. (1875). *The movements and habits of climbing plants*. John Murray.

- Darwin, C., & Darwin, F. (1880). *The power of movement in plants*. John Murray.
- Darwin, C., & Darwin, F. (1888). *Insectivorous plants*. John Murray.
- De Kroons, H., & Hutchings, M. J. (1995). Morphological plasticity in clonal plants: The foraging concept reconsidered. *Journal of Ecology*, 83(1), 143-152.
Doi:10.2307/2261158
- De Loof, A. (2016). The cell's self-generated “electrome”: The biophysical essence of the immaterial dimension of Life?. *Communicative & Integrative Biology*, 9(5), e1197446. Doi:10.1080/19420889.2016.1197446
- de Toledo, G. R., Parise, A. G., Simmi, F. Z., Costa, A. V., Senko, L. G., Debono, M. W., & Souza, G. M. (2019). Plant electrome: the electrical dimension of plant life. *Theoretical and Experimental Plant Physiology*, 31(1), 21-46.
Doi:10.1007/s40626-019-00145-x
- Debono, M. W. (2013). Dynamic protoneural networks in plants: a new approach of spontaneous extracellular potential variations. *Plant Signaling & Behavior*, 8(6), e24207. Doi:10.4161/psb.24207
- Dener, E., Kacelnik, A., & Shemesh, H. (2016). Pea plants show risk sensitivity. *Current Biology*, 26(13), 1763-1767. Doi:10.1016/j.cub.2016.05.008
- Dewey, J. (1896). The reflex arc concept in psychology. *Psychological Review*, 3(1), 357–370. Doi:10.1037/h0070405
- Dewey, J. (1916). *Essays in experimental logic*. University of Chicago Press.
- Dewey, J. (1938). *Logic: the theory of inquiry*. Holt, Rinehart & Winston.
- Di Paolo, E., Rohde, M. & De Jaegher, H. (2008). *Horizons for the enactive mind: values, social interaction, and play*. In: Stewart, J., Gapenne, O. & Di Paolo, E. (Eds.) *Enaction: towards a new paradigm for cognitive science*. MIT Press

- Dietrich, D. (2018). Hydrotropism: how roots search for water. *Journal of Experimental Botany*, 69(11), 2759-2771. Doi:10.1093/jxb/ery034
- Dodd, I. C., Ngo, C., Turnbull, C. G. N. & Beveridge, C. A. (2004). Effects of nitrogen supply on xylem cytokinin delivery, transpiration and leaf expansion of pea genotypes differing in xylem-cytokinin concentration. *Functional Plant Biology*, 31(9), 903–911. Doi:10.1071/FP04044
- Ducatez, S., Audet, J. N., & Lefebvre, L. (2015). Problem-solving and learning in Carib grackles: Individuals show a consistent speed–accuracy trade-off. *Animal Cognition*, 18(2), 485–496. Doi:10.1007/s10071-014-0817-1
- Dudareva, N., & Pichersky, E. (2000). Biochemical and molecular genetic aspects of floral scents. *Plant Physiology*, 122(3), 627-634. Doi:10.1104/pp.122.3.627
- Dudley, S. A., & File, A. L. (2007). Kin recognition in an annual plant. *Biology Letters*, 3(4), 435-438. Doi:10.1098/rsbl.2007.0232
- Dudley, S. A., Murphy, G. P., & File, A. L. (2013). Kin recognition and competition in plants. *Functional Ecology*, 27(4), 898-906. Doi:65-2435.12121
- Durbak, A., Yao, H., & McSteen, P. (2012). Hormone signaling in plant development. *Current Opinion in Plant Biology*, 15(1), 92-96. Doi: 10.1016/j.pbi.2011.12.004
- Eliasson, A. C., Rösblad, B., & Forssberg, H. (2004). Disturbances in programming goal-directed arm movements in children with ADHD. *Developmental Medicine and Child Neurology*, 46(1), 19-27. Doi:10.1017/S0012162204000040
- Engel, A. K. (2010). *Directive minds: how dynamics shapes cognition*. In Stewart, J. et al (Eds.) *Enaction: Towards a new paradigm for cognitive science* (pp. 219-243). MIT Press.

- Engel, A. K., Maye, A., Kurthen, M., & König, P. (2013). Where's the action? The pragmatic turn in cognitive science. *Trends in Cognitive Sciences*, 17(5), 202-209. Doi:10.1016/j.tics.2013.03.006
- Falik, O., Reides, P., Gersani, M., & Novoplansky, A. (2005). Root navigation by self-inhibition. *Plant, Cell and Environment*, 28(4), 562–569. Doi:10.1111/j.1365-3040.2005.01304.x
- Fang, S., Yan, X., & Liao, H. (2009). 3D reconstruction and dynamic modeling of root architecture in situ and its application to crop phosphorus research. *The Plant Journal*, 60(6), 1096-1108. Doi:10.1111/j.1365-313X.2009.04009.x
- Farmer, E. E., & Ryan, C. A. (1990). Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences*, 87(19), 7713-7716. Doi:10.1073/pnas.87.19.7713
- Fitts, P. M. (1954). The information capacity of the human motor system in controlling the amplitude of movement. *Journal of Experimental Psychology*, 47(6), 381–391. Doi:10.1037/h0055392
- Fitts, P. M., & Peterson, J. R. (1964). Information capacity of discrete motor responses. *Journal of Experimental Psychology*, 67(2), 103–112. Doi:10.1037/h0045689
- Fodor, J. A., & Pylyshyn, Z. W. (1981). How direct is visual perception? Some reflections on Gibson's “ecological approach”. *Cognition*, 9(2), 139–196. Doi:10.1016/0010-0277(81)90009-3
- Forde, B. G. (2002). Local and long-range signaling pathways regulating plant responses to nitrate. *Annual Review of Plant Biology*, 53(1), 203-224. Doi:10.1146/annurev.arplant.53.100301.135256

- Fradet, L., Lee, G., & Dounskaia, N. (2008). Origins of submovements during pointing movements. *Acta Psychologica*, *129*(1), 91-100. Doi:10.1016/j.actpsy.2008.04.009
- Franks, N. R., Dornhaus, A., Fitzsimmons, J. P., & Stevens, M. (2003). Speed versus accuracy in collective decision making. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1532), 2457–2463. Doi:10.1098/rspb.2003.2527
- French, A. P., Bennett, M. J., Howells, C., Patel, D., & Pridmore, T. (2008). A probabilistic tracking approach to root measurement in images. *In Proceedings of the First International Conference on Biomedical Electronics and Devices, BIOSIGNALS, 1*, 108-115.
- French, A., Ubeda-Tomás, S., Holman, T. J., Bennett, M. J., & Pridmore, T. (2009). High-throughput quantification of root growth using a novel image-analysis tool. *Plant Physiology*, *150*(4), 1784-1795. Doi:10.1104/pp.109.140558
- Fromm, J., & Fei, H. (1998). Electrical signaling and gas exchange in maize plants of drying soil. *Plant Science*, *132*(2), 203-213. Doi:10.1016/S0168-9452(98)00010-7
- Fromm, J., & Lautner, S. (2007). Electrical signals and their physiological significance in plants. *Plant, Cell & Environment*, *30*(3), 249-257. Doi:10.1111/j.1365-3040.2006.01614.x
- Fukano, Y., & Yamawo, A. (2015). Self-discrimination in the tendrils of the vine *Cayratia japonica* is mediated by physiological connection. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1814), 20151379. Doi:10.1098/rspb.2015.1379
- Gagliano, M. (2013). The flowering of plant bioacoustics: how and why. *Behavioral Ecology*, *24*(4), 800-801. Doi:10.1093/beheco/art021

- Gagliano, M., Grimonprez, M., Depczynski, M., & Renton, M. (2017). Tuned in: Plant roots use sound to locate water. *Oecologia*, *184*(1), 151-160. Doi:10.1007/s00442-017-3862-z
- Gagliano, M., Mancuso, S., & Robert, D. (2012a). Towards understanding plant bioacoustics. *Trends in Plant Science*, *17*(6), 323-325.
Doi:10.1016/j.tplants.2012.03.002
- Gagliano, M., Renton, M., Duvdevani, N., Timmins, M., & Mancuso, S. (2012b). Out of sight but not out of mind: Alternative means of communication in plants. *PLOS ONE*, *7*(5). Doi:10.1371/journal.pone.0037382
- Gagliano, M., Vyazovskiy, V.V., Borbély A. A., Grimonprez, M., & Depczynski, M. (2016). Learning by association in plants. *Scientific Reports*, *6*, 38427.
Doi:10.1038/srep38427
- Gallagher, S. (2005). Phenomenological contributions to a theory of social cognition. *Husserl studies*, *21*(2), 95-110. Doi:10.1007/s10743-005-6402-3
- Gallentine, J., Wooten, M. B., Thielen, M., Walker, I. D., Speck, T., & Niklas, K. (2020). Searching and intertwining: Climbing plants and growbots. *Frontiers in Robotics and AI*, *7*, 118. Doi:10.3389/frobt.2020.00118
- Gandar, P. W. (1983). Growth in root apices. II. Deformation and rate of deformation. *Botanical Gazette*, *144*(1), 11-19. Doi:10.1086/337338
- Garbin, M. L., Carrijo, T. T., Sansevero, J. B. B., Sánchez-Tapia, A. & Scarano, F. R. (2012). Subordinate, not dominant, woody species promote the diversity of climbing plants. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*, 257–265. Doi:10.1016/j.ppees.2012.03.001

- Gavelis, G. S., Hayakawa, S., White III, R. A., Gojobori, T., Suttle, C. A., et al. (2015). Eye-like ocelloids are built from different endosymbiotically acquired components. *Nature*, 523(7559), 204-207. Doi:10.1038/nature14593
- Gehring, W. J. (2005). New perspectives on eye development and the evolution of eyes and photoreceptors. *Journal of Heredity*, 96(3), 171-184. Doi:10.1093/jhered/esi027
- Gentry, A. H., Putz, F. E., & Mooney, H. A. (1991). *The biology of vines*. (pp. 393-423). Editorial Cambridge, Cambridge University Press
- Gianoli, E. (2001). Lack of differential plasticity to shading of internodes and petioles with growth habit in *Convolvulus arvensis* (Convolvulaceae). *International Journal of Plant Sciences*, 162, 1247–1252. Doi:10.1086/322950
- Gianoli, E. (2003). Phenotypic responses of the twining vine *Ipomoea purpurea* (Convolvulaceae) to physical support availability in sun and shade. *Plant Ecology*, 165, 21–26. Doi:10.1023/A:1021412030897
- Gianoli, E. (2015). The behavioural ecology of climbing plants. *AoB PLANTS*, 7, plv013. Doi:10.1093/aobpla/plv013
- Gianoli, E., & Carrasco-Urra, F. (2014). Leaf mimicry in a climbing plant protects against herbivory. *Current Biology*, 24(9), 984–987. Doi:10.1016/j.cub.2014.03.010
- Gibson, J. J. (1979). *The ecological approach to visual perception*. Houghton Mifflin.
- Girloy, S. (2008). Plant tropisms. *Current Biology*, 18(7), R275-R277.
- Gomila, T. & Calvo, P. (2008). *Directions for an embodied cognitive science: towards and integrated approach*. In: Calvo, P. & Gomila, T. (Eds.) *Handbook of cognitive science: an embodied approach* (pp. 1-25). Elsevier.

- Goriely, A., & Neukirch, S. (2006). Mechanics of climbing and attachment in twining plants. *Physical Review Letters*, *97*(18), 184302.
Doi:10.1103/PhysRevLett.97.184302
- Grime, J. P., & Mackey, J. M. L. (2002). The role of plasticity in resource capture by plants. *Evolutionary Ecology*, *16*(3), 299-307. Doi:10.1023/A:1019640813676
- Guo, Y., Chen, F., Zhang, F., & Mi, G. (2005). Auxin transport from shoot to root is involved in the response of lateral root growth to localized supply of nitrate in maize. *Plant Science*, *169*(5), 894-900. Doi:10.1016/j.plantsci.2005.06.007
- Hamant, O., & Moullia, B. (2016). How do plants read their own shapes?. *New Phytologist*, *212*(2), 333-337. Doi:10.1111/nph.14143
- Hammerschmidt, R. & Kuc, J. (1995). *Induced resistance to disease in plants*. Kluwer, Dordrecht: The Netherlands
- Hammond, J. P., & White, P. J. (2011). Sugar signaling in root responses to low phosphorus availability. *Plant Physiology*, *156*(3), 1033-1040.
Doi:10.1104/pp.111.175380
- Harper, J. L. (1977). *Population biology of plants*. Academic Press
- Hartley, R., Zisserman, A. & Faugeras, O. (2004). *Multiple view geometry in computer vision*. Cambridge University Press (CUP): Cambridge, UK.
- Hashiguchi, Y., Tasaka, M., & Morita, M. T. (2013). Mechanism of higher plant gravity sensing. *American Journal of Botany*, *100*(1), 91-100. Doi:10.3732/ajb.1200315
- Hatakeda, Y., Kamada, M., Goto, N., Fukaki, H., Tasaka, M., Suge, H., & Takahashi, H. (2003). Gravitropic response plays an important role in the nutational movements of the shoots of *Pharbitis nil* and *Arabidopsis thaliana*. *Physiologia Plantarum*, *118*(3), 464-473. Doi:10.1034/j.1399-3054.2003.00080.x

- Haupt, W., & Feinleib, M. E. (1979). *Physiology of movements*. In: Pirson, A. & Zimmermann, M. H. (Eds.). *Encyclopedia of plant physiology*. Springer-Verlag.
- Heathcote, D. G., & Aston, T. J. (1970). The physiology of plant nutation: i. nutation and geotropic response. *Journal of Experimental Botany*, *21*(4), 997-1002.
Doi:10.1093/jxb/21.4.997
- Hegarty, E. E. (1991). *Vine–host interactions*. In: Putz, F. E. & Mooney, H. A. (Eds.). *The biology of vines* (pp. 357-375). Cambridge University Press.
- Heil, M., & Karban, R. (2010). Explaining evolution of plant communication by airborne signals. *Trends in Ecology & Evolution*, *25*(3), 137-144.
Doi:10.1016/j.tree.2009.09.010
- Heil, M., & Ton, J. (2008). Long-distance signalling in plant defence. *Trends in Plant Science*, *13*(6), 264-272. Doi:10.1016/j.tplants.2008.03.005
- Heitz, R. P., & Schall, J. D. (2012). Neural mechanisms of speed–accuracy trade-off. *Neuron*, *76*(3), 616–628. Doi:10.1016/j.neuron.2012.08.030
- Hille, B. (1992). *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer Associates.
- Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist*, *162*(1), 9-24. Doi:10.1111/j.1469-8137.2004.01015.x
- Hodge, A. (2009). Root decisions. *Plant, Cell & Environment*, *32*(6), 628-640.
Doi:10.1111/j.1365-3040.2008.01891.x
- Hodge, A., Berta, G., Doussan, C., Merchan, F., & Crespi, M. (2009). Plant root growth, architecture and function. *Plant and Soil*, *321*(1), 153-187. Doi:10.1007/s11104-009-9929-9
- Hooker, H. D. (1915). Hydrotropism in roots of *Lupinus albus*. *Annals of Botany*, *29*(114), 265-283.

- Huber, A. E., & Bauerle, T. L. (2016). Long-distance plant signaling pathways in response to multiple stressors: the gap in knowledge. *Journal of Experimental Botany*, 67(7), 2063-2079. Doi:10.1093/jxb/erw099
- Huey, R. B., Carlson, M., Crozier, L., Frazier, M., Hamilton, H., Harley, C. et al. (2002). Plants versus animals: do they deal with stress in different ways?. *Integrative and Comparative Biology*, 42(3), 415-423. Doi:10.1093/icb/42.3.415
- Hurley, S. L. (1998). Vehicles, contents, conceptual structure, and externalism. *Analysis*, 58(1), 1-6.
- Hutchings, M. J., & de Kroon, H. (1994). Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research*, 25, 159-238. Doi:10.1016/S0065-2504(08)60215-9
- Inoue, N., Arase, T., Hagiwara, M., Amnao, T., Hyashi, T. & Ikeda, R. (1999). Ecological significance of root tip rotation for seedling establishment of *Oryza sativa* L.. *Ecological Research*, 14(1), 31-8
- Israelsson, D. & Johnsson, A. (1967). A theory for circumnutations in *Helianthus annuus*. *Physiologia Plantarum*, 20, 957-76.
- Jacobs, M. R. (1954). The effect of wind sway on the form and development of *Pinus radiata* D. Don. *Australian Journal of Botany*, 2(1), 35-51. Doi:10.1071/BT9540035
- Jacobson, S. L. (1965). Receptor response in Venus's fly-trap. *The Journal of General Physiology*, 49(1), 117-129. Doi:10.1085/jgp.49.1.117
- Jaffe, M. J. (1980). *On the mechanism of contact coiling of tendrils*. In Plant Growth Substances 1979. (pp. 481-495). Springer. Doi:10.1007/978-3-642-67720-5_49

- Jaffe, M. J., & Galston, A. W. (1968b). Physiological studies on pea tendrils. V. Membrane changes and water movement associated with contact coiling. *Plant Physiology*, 43, 537-542. Doi:10.1104/pp.43.4.537
- Jaffe, M. J., & Galston, A. W. (1967a). Physiological studies on pea tendrils. III. ATPase activity and contractility associated with coiling. *Plant Physiology*, 42(6), 845-847. Doi:10.1104/pp.42.6.845
- Jaffe, M. J., & Galston, A. W. (1967b). Physiological studies on pea tendrils. IV. Flavonoids and contact coiling. *Plant Physiology*, 42(6), 848-850. Doi:10.1104/pp.42.6.848
- Jaffe, M. J., & Galston, A. W. (1967c). Phytochrome control of rapid nyctinastic movements and membrane permeability in *Albizia julibrissin*. *Planta*, 77(2), 135-141. Doi:10.1007/BF00387450
- Jaffe, M. J., & Galston, A. W. (1968a). The physiology of tendrils. *Annual Review of Plant Physiology*, 19, 417-434. Doi:10.1146/annurev.pp.19.060168.002221
- Jaffe, M. J., Takahashi, H., & Biro, R. L. (1985). A pea mutant for the study of hydrotropism in roots. *Science*, 230(4724), 445-447. Doi:10.1126/science.230.4724.445
- Japyassú, H. F., & Laland, K. N. (2017). Extended spider cognition. *Animal Cognition*, 20(3), 375-395. Doi:10.1007/s10071-017-1069-7
- Jeannerod, M. (1984). The timing of natural prehension movements. *Journal of Motor Behavior*, 16(3), 235-254. Doi:10.1080/00222895.1984.10735319
- Joerrens, G. (1959). Nutationsbewegungen bei *Triticum-Koleoptilen*. *Z. f. Bot.* 47, 403-420
- Johnsson, A. (1979). *Circumnutation*. In: Haupt, W. & Feinleib, E. (Eds.) *Encyclopedia of plant physiology, Physiology of Movements* (pp. 627-46).

- Johnsson, A., & Israelsson, D. (1968). Application of a theory for circumnutations to geotropic movements. *Physiologia Plantarum*, *21*(2), 282-291.
Doi:10.1111/j.1399-3054.1968.tb07251.x
- Johnsson, A., Haupt, W. & Feinleib, E. (1979). *Circumnutation. Encyclopedia of plant physiology, Physiology of Movements* (pp. 627-646). Springer.
- Johnsson, A., Jansen, C., Engelmann, W., & Schuster J. (1999). Circumnutations without gravity: a two-oscillator model. *Journal of Gravitational Physiology*, *6*, 9-12.
- Johnsson, A., Solheim, B. G. B., & Iversen, T. H. (2009). Gravity amplifies and microgravity decreases circumnutations in *Arabidopsis thaliana* stems: results from a space experiment. *New Phytologist*, *182*(3), 621-629. Doi:10.1111/j.1469-8137.2009.02777.x
- Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323-329. Doi:10.1038/nature05286
- Kacelnik, A., & Bateson, M. (1997). Risk-sensitivity: Crossroads for theories of decision-making. *Trends in Cognitive Science*, *1*, 304-309, Doi:10.1016/S1364-6613(97)01093-0.
- Kaplan, D. M. (2012). How to demarcate the boundaries of cognition. *Biology & Philosophy*, *27*(4), 545-570. Doi:10.1007/s10539-012-9308-4
- Karban, R. (2008). Plant behaviour and communication. *Ecology Letters*, *11*(7), 727-739. Doi:10.1111/j.1461-0248.2008.01183.x
- Karban, R. (2015). *Plant sensing and communication*. Chicago, IL: University of Chicago Press.
- Karban, R., & Shiojiri, K. (2009). Self-recognition affects plant communication and defense. *Ecology Letters*, *12*(6), 502-506. Doi:10.1111/j.1461-0248.2009.01313.x

- Karban, R., Huntzinger, M., & McCall, A. C. (2004). The specificity of eavesdropping on sagebrush by other plants. *Ecology*, *85*(7), 1846-1852. Doi:10.1890/03-0593
- Karban, R., Shiojiri, K., & Ishizaki, S. (2011). Plant communication—why should plants emit volatile cues?. *Journal of Plant Interactions*, *6*(2-3), 81-84.
Doi:10.1080/17429145.2010.536589
- Karban, R., Wetzel, W. C., Shiojiri, K., Ishizaki, S., Ramirez, S. R., & Blande, J. D. (2014). Deciphering the language of plant communication: volatile chemotypes of sagebrush. *New Phytologist*, *204*(2), 380-385. Doi:10.1111/nph.12887
- Keijzer, F. A. (2017). Evolutionary convergence and biologically embodied cognition. *Interface Focus*, *7*, 20160123. Doi:10.1098/rsfs.2016.0123
- Kelly, C. K. (1990). Plant foraging: a marginal value model and coiling response in *Cuscuta subinclusa*. *Ecology*, *71*(5), 1916-1925. Doi:10.2307/1937599
- Khait, I., Obolski, U., Yovel, Y., & Hadany, L. (2019). Sound perception in plants. *Seminars in Cell & Developmental Biology*, *92*, 134-138.
Doi:10.1016/j.semcdb.2019.03.006
- Kiss, J. Z. (2006). Up, down, and all around: How plants sense and respond to environmental stimuli. *Proceedings of the National Academy of Sciences, USA*, *103*(4), 829–830. Doi:10.1073/pnas.0510471102
- Kitazawa, D., Hatakeda, Y., Kamada, M., Fujii, N., Miyazawa, Y., Hoshino, A., et al. (2005). Shoot circumnutation and winding movements require gravisensing cells. *Proceedings of the National Academy of Science of the United States of America*, *102*, 18742-7. Doi:10.1073/pnas.0504617102
- Knight, T. A. (1806). V. On the direction of the radicle and germen during the vegetation of seeds. By Thomas Andrew knight, Esq. FRS In a letter to the right Hon. Sir

- Joseph banks, KBPR S. *Philosophical Transactions of the Royal Society of London*, 96, 99-108.
- Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H. et al. (2020). Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proceedings of the National Academy of Sciences*, 117(7), 3874-3883. Doi:10.1073/pnas.1912130117
- Kosuge, K., Iida, S., Katou, K., & Mimura, T. (2013). Circumnutation on the water surface: female flowers of *Vallisneria*. *Scientific Reports*, 3(1), 1-7. Doi:10.1038/srep01133
- Kozlowski, T. T. (1999). Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research*, 14(6), 596-619. Doi:10.1080/02827589908540825
- Kozlowski, T. T. (1999). Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research*, 14(6), 596-619. Doi:10.1080/02827589908540825
- Kruschke, J. K. (2013). Bayesian estimation supersedes the t test. *Journal of Experimental Psychology: General*, 142(2), 573–603. Doi:10.1037/a0029146
- Kruschke, J. K., & Meredith, M. (2018). *BEST: Bayesian estimation supersedes the t-test* (R Package Version 0.5.0) [Computer software]. Retrieved from <http://CRAN.R-project.org/package=BEST>
- Lee, H. J., Kim, H. S., Park, J. M., Cho, H. S., & Jeon, J. H. (2020). PIN-mediated polar auxin transport facilitates root– obstacle avoidance. *New Phytologist*, 225(3), 1285-1296. Doi:10.1111/nph.16076
- Leopold, A. C., Jaffe, M. J., Brokaw, C. J. & Goebe. G. (2000). Many modes of movement. *Science*, 288(5474), 2131–2132. Doi:10.1126/science.288.5474.2131e
- Leyser, O., & Day, S. (2003). *Mechanisms in Plant Development*. Blackwell Science, Oxford, UK.

- Li, X., & Zhang, W. S. (2008). Salt-avoidance tropism in *Arabidopsis thaliana*. *Plant Signaling & Behavior*, 3(5), 351-353. Doi:10.4161/psb.3.5.5371
- Lucas, B. D., & Kanade, T. (1981). *An iterative image registration technique with an application to stereo vision*. In Proceedings of the 7th Int. Joint Conf. on Artificial Intelligence (IJCAI), Vancouver, BC, USA, 24–28 April 1981; pp. 674–679.
- Lüttge, U. (2021). Integrative emergence in contrast to separating modularity in plant biology: views on systems biology with information, signals and memory at scalar levels from molecules to the biosphere. *Theoretical and Experimental Plant Physiology*, 33(1), 1-13. Doi:10.1007/s40626-021-00198-x
- Lynch, J. P., Nielsen, K. L., Davis, R. D., & JablOKow, A. G. (1997). SimRoot: modelling and visualization of root systems. *Plant and Soil*, 188(1), 139-151.
Doi:10.1023/A:1004276724310
- Lyon, P. (2015). The cognitive cell: bacterial behavior reconsidered. *Frontiers in Microbiology*, 6, 264. Doi:10.3389/fmicb.2015.00264
- MacDougal, D. T. (1893). Inter-Twining of Tendrils. *Botanical Gazette*, 18(10), 396-397.
- Mahall, B. E., & Callaway, R. M. (1992). Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology*, 73(6), 2145-2151. Doi:10.2307/1941462
- Mairhofer, S., Zappala, S., Tracy, S. R., Sturrock, C., Bennett, M., Mooney, S. J., & Pridmore, T. (2012). RooTrak: automated recovery of three-dimensional plant root architecture in soil from X-ray microcomputed tomography images using visual tracking. *Plant Physiology*, 158(2), 561-569. Doi:10.1104/pp.111.186221
- Malone, M. (1994). Wound-induced hydraulic signals and stimulus transmission in *Mimosa pudica* L. *New Phytologist*, 128(1), 49-56. Doi:10.1111/j.1469-8137.1994.tb03985.x

- Mancuso, S., & Viola, A. (2013). *Verde Brillante: Sensibilità e intelligenza del mondo vegetale*. Giunti.
- Marr, D. (1982). *Vision: A computational investigation into the human representation and processing of visual information*. W.H. Freeman.
- Massa, G. D., & Gilroy, S. (2003). Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. *The Plant Journal*, 33(3), 435-445.
Doi:10.1046/j.1365-313X.2003.01637.x
- Maturana, H. R., & Varela, F. J. (1980). *Problems in the neurophysiology of cognition*. In *Autopoiesis and cognition* (pp. 41-47). Springer.
- McGann, M. (2007). Enactive theorists do it on purpose: Toward an enactive account of goals and goal-directedness. *Phenomenology and the Cognitive Sciences*, 6(4), 463-483. Doi:10.1007/s11097-007-9074-y
- McNamara, J. M., & Houston, A. I. (1992). Risk-sensitive foraging: a review of the theory. *Bulletin of Mathematical Biology*, 54(2-3), 355-378.
- McNickle, G. G., Clair, C. C. S., & Cahill Jr, J. F. (2009). Focusing the metaphor: plant root foraging behaviour. *Trends in Ecology & Evolution*, 24(8), 419-426.
Doi:10.1016/j.tree.2009.03.004
- Merleau-Ponty, M. (1945). *Phenomenology of perception*. Routledge.
- Merleau-Ponty, M. (1963). Elogio de Filosofia. *Revista Portuguesa de Filosofia*, 19(1), 93.
- Mescher, M. C., Runyon, J., & De Moraes, C. M. (2006). Plant host finding by parasitic plants: a new perspective on plant to plant communication. *Plant Signaling & Behavior*, 1(6), 284-286. Doi:10.4161/psb.1.6.3562

- Meyer, D. E., Abrams, R. A., Kornblum, S., Wright, C. E., & Smith, J. E. K. (1988).
Optimality in human motor performance: Ideal control of rapid aimed movements.
Psychological Review, 95(3), 340–370. Doi:10.1037/0033-295x.95.3.340
- Migliaccio, F., & Piconese, S. (2001). Spiralizations and tropisms in Arabidopsis roots.
Trends in Plant Science, 6(12), 561-565. Doi:10.1016/S1360-1385(01)02152-5
- Migliaccio, F., Tassone, P., & Fortunati, A. (2013). Circumnutation as an autonomous root
movement in plants. *American Journal of Botany*, 100(1), 4-13.
Doi:10.3732/ajb.1200314
- Millet, B., Melin, D. & Badot, P. M. (1988). Circumnutation in *Phaseolus vulgaris*. I.
Growth, osmotic potential and cell ultrastructure in the free-moving part of the
shoot. *Physiologia Plantarum*, 72, 133–138. Doi:10.1111/j.1399-
3054.1988.tb06634.x
- Minorsky, P. V. (2003). Agricultural Importance of Circumnutation. *Plant Physiology*,
132(4), 1779–1780. Doi:10.1104/pp.900085
- Mirza, J. I. (1987). The effects of light and gravity on the horizontal curvature of roots of
gravitropic and agravitropic *Arabidopsis thaliana* L. *Plant Physiology*, 83(1), 118-
120. Doi:10.1104/pp.83.1.118
- Mishra, R. C., Ghosh, R., & Bae, H. (2016). Plant acoustics: in the search of a sound
mechanism for sound signaling in plants. *Journal of Experimental Botany*, 67(15),
4483-4494. Doi:10.1093/jxb/erw235
- Mizuno, H., Kobayashi, A., Fujii, N., Yamashita, M., & Takahashi, H. (2002).
Hydrotropic response and expression pattern of auxin-inducible gene, CS-IAA1, in
the primary roots of clinorotated cucumber seedlings. *Plant and Cell Physiology*,
43(7), 793-801. Doi:10.1093/pcp/pcf093

- Molina-Freaner, F., & Tinoco-Ojanguren, C. (1997). Vines of a desert plant community in Central Sonora, Mexico. *Biotropica*, 29(1), 46-56. Doi:10.1111/j.1744-7429.1997.tb00005.x
- Montagu, K. D., Conroy, J. P., & Francis, G. S. (1998). Root and shoot response of field-grown lettuce and broccoli to a compact subsoil. *Australian Journal of Agricultural Research*, 49(1), 89-98. Doi:10.1071/A97051
- Morita, M. T. (2010). Directional gravity sensing in gravitropism. *Annual Review of Plant Biology*, 61, 705-720. Doi:10.1146/annurev.arplant.043008.092042
- Morita, M. T., & Tasaka, M. (2004). Gravity sensing and signaling. *Current Opinion in Plant Biology*, 7(6), 712-718. Doi:10.1016/j.pbi.2004.09.001
- Mottet, D., & Bootsma, R. J. (1999). The dynamics of goal-directed rhythmical aiming. *Biological Cybernetics*, 80(4), 235-245. Doi:10.1007/s004220050521
- Mouliia, B., Bastien, R., Chauvet-Thiry, H., & Leblanc-Fournier, N. (2019). Posture control in land plants: growth, position sensing, proprioception, balance, and elasticity. *Journal of Experimental Botany*, 70(14), 3467-3494. Doi:10.1088/1748-3190/ab30d3
- Mouliia, B., Douady, S., & Hamant, O. (2021). Fluctuations shape plants through proprioception. *Science*, 372(6540). Doi:10.1126/science.abc6868
- Mugnai S., Azzarello E., Masi E., Pandolfi C. & Mancuso S. (2007). *Nutation in Plants*. In: Mancuso S. & Shabala, S. (Eds.) Rhythms in Plants. Springer. Doi:10.1007/978-3-540-68071-0_4
- Mullen, J. L., Turk, E., Johnson, K., Wolverson, C., Ishikawa, H., Simmons, C. et al. (1998). Root-growth behavior of the Arabidopsis mutant rgr1: roles of gravitropism and circumnutation in the waving/coiling phenomenon. *Plant Physiology*, 118(4), 1139-1145. Doi:10.1104/pp.118.4.1139

- Müller, L. M., von Korff, M., & Davis, S. J. (2014). Connections between circadian clocks and carbon metabolism reveal species-specific effects on growth control. *Journal of Experimental Botany*, *65*(11), 2915-2923. Doi:10.1093/jxb/eru117
- Mumm, R., Schrank, K., Wegener, R., Schulz, S., & Hilker, M. (2003). Chemical analysis of volatiles emitted by *Pinus sylvestris* after induction by insect oviposition. *Journal of Chemical Ecology*, *29*(5), 1235-1252. Doi:10.1023/A:1023841909199
- Nabe-Nielsen, J. (2001). Diversity and distribution of lianas in a neotropical rain forest, Yasuní National Park, Ecuador. *Journal of Tropical Ecology*, *17*(1), 1-19. Doi:10.1017/S0266467401001018
- Nakamura, M., Nishimura, T., & Morita, M. T. (2019). Gravity sensing and signal conversion in plant gravitropism. *Journal of Experimental Botany*, *70*(14), 3495-3506. Doi:10.1016/j.pbi.2019.07.005
- Nakata, K. (2010). Attention focusing in a sit-and-wait forager: a spider controls its prey-detection ability in different web sectors by adjusting thread tension. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1678), 29-33. Doi:10.1098/rspb.2009.1583
- Nakata, K. (2013). Spatial learning affects thread tension control in orb-web spiders. *Biology Letters*, *9*(4), 20130052. Doi:10.1098/rsbl.2013.0052
- Neisser, U. (1967). *Cognitive psychology*. East Norwalk, CT: Appleton-Century-Crofts.
- Ney, D., & Pilet, P. E. (1981). Nutation of growing and georsacting roots. *Plant, Cell & Environment*, *4*(4), 339-343. Doi:10.1111/1365-3040.ep11604564
- Noë, A. (2006). Experience without the head. *Perceptual Experience*, *1*, 411-433.
- Notaguchi, M., & Okamoto, S. (2015). Dynamics of long-distance signaling via plant vascular tissues. *Frontiers in Plant Science*, *6*, 161. Doi:10.3389/ fpls.2015.00161

- Novak, K. E., Miller, L. E., & Houk, J. C. (2002). The use of overlapping submovements in the control of rapid hand movements. *Experimental Brain Research*, 144(3), 351-364. Doi:10.1007/s00221-002-1060-6
- Nozue, K., Covington, M. F., Duek, P. D., Lorrain, S., Fankhauser, C., Harmer, S. L., & Maloof, J. N. (2007). Rhythmic growth explained by coincidence between internal and external cues. *Nature*, 448(7151), 358-361. Doi:10.1038/nature05946
- O'regan, J. K., & Noë, A. (2001). A sensorimotor account of vision and visual consciousness. *Behavioral and Brain Sciences*, 24(5), 939-973. Doi:10.1017/S0140525X01000115
- Okada, K., & Shimura, Y. (1990). Reversible root tip rotation in Arabidopsis seedlings induced by obstacle-touching stimulus. *Science*, 250(4978), 274-276. Doi:10.1126/science.250.4978.274
- Okamoto, K., Ueda, H., Shimada, T., Tamura, K., Kato, T., Tasaka, M., et al. (2015). Regulation of organ straightening and plant posture by an actin–myosin XI cytoskeleton. *Nature plants*, 1(4), 1-7. Doi:10.1038/nplants.2015.31
- Orbovic, V. & Poff, K. L. (1997). Interaction of light and gravitropism with nutation of hypocotyls of Arabidopsis thaliana seedlings. *Plant Growth Regulation*, 23(3), 141-6. Doi:10.1023/a:1005853128971
- Palm, L. H. (1827). *Über das winden der Pflanzen*. C. Richter.
- Parise, A. G., Gagliano, M., & Souza, G. M. (2020). Extended cognition in plants: Is it possible? *Plant Signaling & Behavior*, 1710661. Doi:10.1080/15592324.2019.1710661
- Parise, A. G., Reissig, G. N., Basso, L. F., Senko, L. G. S., Oliveira, T. F. D. C., de Toledo, G. R. A., et al. (2021). Detection of different hosts from a distance alters

- the behaviour and bioelectrical activity of *Cuscuta racemosa*. *Frontiers in Plant Science*, 12, 409. Doi:10.3389/fpls.2021.594195
- Paul, A. L., Amalfitano, C. E., & Ferl, R. J. (2012). Plant growth strategies are remodeled by spaceflight. *BMC Plant Biology*, 12(1), 1-15. Doi:10.1186/1471-2229-12-232
- Paul, G. S., & Yavitt, J. B. (2011). Tropical vine growth and the effects on forest succession: a review of the ecology and management of tropical climbing plants. *The Botanical Review*, 77(1), 11-30. Doi:10.1007/s12229-010-9059-3
- Pavlov, I. (1927). *Uslovnyj rflex.* Moscow: M.G.U (trad. it. I riflessi condizionati. Torino: Boringhieri, 1977).
- Peirce, C. S. (1887). Logical Machines. Modern Logic. *The American Journal of Psychology*. 1(1), 165–170.
- Peñalosa, J. (1982). Morphological specialization and attachment success in two twining lianas. *American Journal of Botany*, 69(6), 1043–1045. Doi:10.1002/j.1537-2197.1982.tb13348.x
- Pfeffer, S. E., Bolek, S., Wolf, H., & Wittlinger, M. (2015). Nest and food search behaviour in desert ants, *Cataglyphis*: a critical comparison. *Animal Cognition*, 18(4), 885-894. Doi:10.1007/s10071-015-0858-0
- Piconese, S., Tronelli, G., Pippia, P., & Migliaccio, F. (2003). Chiral and non-chiral nutations in *Arabidopsis* roots grown on the random positioning machine. *Journal of Experimental Botany*, 54(389), 1909-1918. Doi:10.1093/jxb/erg206
- Pieterse, C. M., van der Does, D., Zamioudis, C., Leon-Reyes, A., & van Wees, S. C. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, 28, 489-521. Doi:10.1146/annurev-cellbio-092910-154055

- Plamondon, R., & Alimi, A. M. (1997). Speed/accuracy trade-offs in target-directed movements. *Behavioral and Brain Sciences*, 20(2), 279–303.
Doi:10.1017/s0140525x97001441
- Porat, A., Tedone, F., Palladino, M., Marcati, P., & Meroz, Y. (2020). A general 3d model for growth dynamics of sensory-growth systems: From plants to robotics. *Frontiers in Robotics and AI*, 7, 89. Doi:10.3389/frobt.2020.00089
- Proffit, M., Khallaf, M. A., Carrasco, D., Larsson, M. C., & Anderson, P. (2015). ‘Do you remember the first time?’ Host plant preference in a moth is modulated by experiences during larval feeding and adult mating. *Ecology Letters*, 18(4), 365–374. Doi:10.1111/ele.12419
- Putz, F. E. (1984). The natural history of lianas on Barro Colorado Island, Panama. *Ecology*, 65(6), 1713–1724. Doi:10.2307/1937767
- Putz, F. E., & Chai P. (1987). Ecological studies of lianas in Lambir National Park, Sarawak, Malaysia. *The Journal of Ecology*, 75, 523–531. Doi:10.2307/2260431
- Putz, F., & Holbrook, N. (1992). *Biomechanical studies of vines*. In F. Putz & H. Mooney (Eds.), *The biology of vines* (pp. 73–98). Cambridge University Press.
Doi:10.1017/CBO9780511897658.005
- R Core Team. (2014). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org>
- Raja, V., Silva, P. L., Holghoomi, R., & Calvo, P. (2020). The dynamics of plant nutation. *Scientific Reports*, 10(1), 1–13. Doi:10.1038/s41598-020-76588-z
- Rodrigo-Moreno, A., Bazihizina, N., Azzarello, E., Masi, E., Tran, D., Bouteau, F., et al. (2017). Root phonotropism: early signalling events following sound perception in *Arabidopsis* roots. *Plant Science*, 264, 9–15. Doi:10.1016/j.plantsci.2017.08.001

- Rosenthal, R. (1991). *Meta-analytic procedures for social research*. 2nd ed. (Sage, Newbury Park, CA).
- Rowe, N. P., Isnard, S., Gallenmüller, F., & Speck, T. (2006). *Diversity of mechanical architectures in climbing plants: An ecological perspective*. In A. Herrel, T. Speck, & N. P. Rowe (Eds.), *Ecology and biomechanics: A mechanical approach to the ecology of animals and plants* (pp. 35–59). CRC Press.
- Runyon, J. B., Mescher, M. C., & De Moraes, C. M. (2006). Volatile chemical cues guide host location and host selection by parasitic plants. *Science*, *313*(5795), 1964–1967. Doi: 10.1126/science.1131371
- Russino, A., Ascrizzi, A., Popova, L., Tonazzini, A., Mancuso, S., & Mazzolai, B. (2013). A novel tracking tool for the analysis of plant-root tip movements. *Bioinspiration & Biomimetics*, *8*(2), 025004. Doi:10.1088/1748-3182/8/2/025004
- Sartori, L., Camperio-Ciani, A. C., Bulgheroni, M., & Castiello, U. (2013). Reaching and grasping behavior in *Macaca fascicularis*: a kinematic study. *Experimental Brain Research*, *224*(1), 119-124. Doi:10.1007/s00221-012-3294-2
- Sartori, L., Camperio-Ciani, A., Bulgheroni, M., & Castiello, U. (2014). How posture affects macaques' reach-to-grasp movements. *Experimental Brain Research*, *232*(3), 919-925. Doi:10.1007/s00221-013-3804-x
- Schuster, J., & Engelmann, W. (1997). Circumnutations of *Arabidopsis thaliana* seedlings. *Biological Rhythm Research*, *28*(4), 422-440. Doi:10.1076/brhm.28.4.422.13117
- Segundo-Ortin, M., & Calvo, P. (2019). Are plants cognitive? A reply to Adams. *Studies in History and Philosophy of Science Part A*, *73*, 64–71.
Doi:10.1016/j.shpsa.2018.12.001

- Segundo-Ortin, M., Heras-Escribano, M., & Raja, V. (2019). Ecological psychology is radical enough: a reply to radical enactivists. *Philosophical Psychology*, 32(7), 1001-1023. Doi:10.1080/09515089.2019.1668238
- Semchenko, M., Saar, S., & Lepik, A. (2014). Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. *New Phytologist*, 204(3), 631-637. Doi:10.1111/nph.12930
- Semchenko, M., Zobel, K., Heinemeyer, A., & Hutchings, M. J. (2008). Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. *New Phytologist*, 179(4), 1162-1170. Doi:10.1111/j.1469-8137.2008.02543.x
- Severino, L. S. (2021). Plants make smart decisions in complex environments. *Plant Signaling & Behavior*, 16(11), 1970448. Doi:10.1080/15592324.2021.1970448
- Shabala, S. (2003). Regulation of potassium transport in leaves: from molecular to tissue level. *Annals of Botany*, 92(5), 627-634. Doi:10.1093/aob/mcg191
- Shabala, S. N., & Newman, I. A. (1997). H⁺ flux kinetics around plant roots after short-term exposure to low temperature: identifying critical temperatures for plant chilling tolerance. *Plant, Cell & Environment*, 20(11), 1401-1410. Doi:10.1046/j.1365-3040.1997.d01-35.x
- Sherrington, C. S. (1907). On the proprio-ceptive system, especially in its reflex aspect. *Brain*, 29, 467-482. Doi:10.1093/brain/29.4.467
- Shiojiri, K., & Karban, R. (2006). Plant age, communication, and resistance to herbivores: young sagebrush plants are better emitters and receivers. *Oecologia*, 149(2), 214-220. Doi:10.1007/s00442-006-0441-0

- Silk, W. K., & Holbrook, N. M. (2005). The importance of frictional interactions in maintaining the stability of the twining habit. *American Journal of Botany*, *92*(11), 1820-1826. Doi:10.3732/ajb.92.11.1820
- Simmons, C., Migliaccio, F., Masson, P., Caspar, T., & Söll, D. (1995a). A novel root gravitropism mutant of *Arabidopsis thaliana* exhibiting altered auxin physiology. *Physiologia Plantarum*, *93*(4), 790-798. Doi:10.1111/j.1399-3054.1995.tb05133.x
- Simmons, C., Söll, D., & Migliaccio, F. (1995b). Circumnutation and gravitropism cause root waving in *Arabidopsis thaliana*. *Journal of Experimental Botany*, *46*(1), 143-150. Doi:10.1093/jxb/46.1.143
- Simojoki, A. (2001). Morphological responses of barley roots to soil compaction and modified supply of oxygen. *Agricultural and Food Science in Finland*, *10*(1), 45–52. Doi:10.23986/afsci.5678
- Simonetti, V., Bulgheroni, M., Guerra, S., Peressotti, A., Peressotti, F., Baccinelli, W., et al. (2021). Can plants move like animals? a three-dimensional stereovision analysis of movement in plants. *Animals*, *11*(7), 1854. Doi:10.3390/ani11071854
- Simons, P. (1992). *The action plant*. Blackwell.
- Simons, P. J. (1981). The role of electricity in plant movements, *New Phytologist*, *87*, 11-37. Doi:10.1111/j.1469-8137.1981.tb01687.x
- Sorensen, A. E. (1986). Seed dispersal by adhesion. *Annual Review of Ecology and Systematics* *17*, 443–463. Doi:10.1146/annurev.es.17.110186.002303
- Souza, G. M. & Lüttge, U. (2015). *Stability as a phenomenon emergent from plasticity-complexity-diversity in eco-physiology*. In: Lüttge, U. & Beyschlag, W. (Eds.) *Progress in botany* (pp. 211-239). Springer Science and Business Media.
- Souza, G. M., Ribeiro R. V., Prado, C. H. B. A., Damineli, D. S. C., Sato, A. M., & Oliveira, M. S. (2009). Using network connectance and autonomy analyses to

- uncover patterns of photosynthetic responses in tropical woody species. *Ecological Complexity*, 6(1), 15–26. Doi:10.1016/j.ecocom.2008.10.002.
- Souza G. M., Toledo G. R. A., & Saraiva G. F. R. (2018). *Towards Systemic View for Plant Learning: Ecophysiological Perspective*. In: Baluska, F., Gagliano, M., & Witzany, G. (Eds) *Memory and Learning in Plants. Signaling and Communication in Plants*. Springer, Cham. Doi:10.1007/978-3-319-75596-0_9
- Spurný, M. (1966). Spiral feedback oscillations of growing hypocotyl with radicle in *Pisum sativum* L.. *Biologia Plantarum*, 8(5), 381. Stahlberg, R. (2006). Historical overview on plant neurobiology. *Plant Signaling & Behavior*, 1(1), 6-8. Doi:10.4161/psb.1.1.2278
- Stahlberg, R., & Cosgrove, D. J. (1992). Rapid alterations in growth rate and electrical potentials upon stem excision in pea seedlings. *Planta*, 187(4), 523-531. Doi:10.1007/BF00199972
- Stahlberg, R., & Cosgrove, D. J. (1996). Induction and ionic basis of slow wave potentials in seedlings of *Pisum sativum* L. *Planta*, 200(4), 416-425. Doi:10.1007/BF00231397
- Stahlberg, R., & Cosgrove, D. J. (1997). The propagation of slow wave potentials in pea epicotyls. *Plant Physiology*, 113(1), 209-217. Doi:10.1104/pp.113.1.209
- Stolarz, M. (2009). Circumnutation as a visible plant action and reaction. *Plant Signaling and Behavior*, 4(5), 380–387. Doi:10.4161/psb.4.5.8293
- Stolarz, M., Dziubinska, H., Krupa, M., Buda, A., Trebacz, K., & Zawadzki, T. (2003). Disturbances of stem circumnutations evoked by wound-induced variation potentials in *Helianthus annuus* L. *Cellular and Molecular Biology Letters*, 8(1), 31-40.

- Stolarz, M., Żuk, M., Król, E., & Dziubińska, H. (2014). Circumnutation Tracker: novel software for investigation of circumnutation. *Plant Methods*, *10*(1), 1-9.
Doi:10.1186/1746-4811-10-24
- Strong Jr, D. R., & Ray Jr, T. S. (1975). Host tree location behavior of a tropical vine (m on sters gi gont ea) by s kototropism. *Science*, *190*, 804-806.
Doi:10.1126/science.190.4216.804
- Su, S. H., Gibbs, N. M., Jancewicz, A. L., & Masson, P. H. (2017). Molecular mechanisms of root gravitropism. *Current Biology*, *27*(17), R964-R972.
Doi:10.1016/j.cub.2017.07.015
- Symons, G. M., & Reid, J. B. (2004). Brassinosteroids do not undergo long-distance transport in pea. Implications for the regulation of endogenous brassinosteroid levels. *Plant Physiology*, *135*(4), 2196-2206. Doi:10.1104/ pp.104.043034
- Takahashi, H., & Suge, H. (1991). Root hydrotropism of an agravitropic pea mutant, ageotropum. *Physiologia Plantarum*, *82*(1), 24-31. Doi:10.1111/j.1399-3054.1991.tb02898.x
- Takahashi, N., Goto, N., Okada, K., & Takahashi, H. (2002). Hydrotropism in abscisic acid, wavy, and gravitropic mutants of *Arabidopsis thaliana*. *Planta*, *216*(2), 203-211. Doi:10.1007/s00425-002-0840-3
- Takahashi, N., Yamazaki, Y., Kobayashi, A., Higashitani, A., & Takahashi, H. (2003). Hydrotropism interacts with gravitropism by degrading amyloplasts in seedling roots of *Arabidopsis* and radish. *Plant Physiology*, *132*(2), 805-810.
Doi:10.1104/pp.018853
- Takei, K., Sakakibara, H., Taniguchi, M., & Sugiyama, T. (2001). Nitrogen-dependent accumulation of cytokinins in root and thetranslocation to leaf: Implication of

- cytokinin species that induces gene expression of maize response regulator. *Plant and Cell Physiology*, 42(1), 85-93. Doi:10.1093/pcp/pce009
- Takei, K., Ueda, N., Aoki, K., Kuromori, T., Hirayama, T., Shinozaki, K. et al. (2004). AtIPT3 is a key determinant of nitrate-dependent cytokinin biosynthesis in *Arabidopsis*. *Plant and Cell Physiology*, 45(8), 1053-1062. Doi:10.1093/pcp/pch119
- Taylor, I., Lehner, K., McCaskey, E., Nirmal, N., Ozkan-Aydin, Y., Murray-Cooper, M., et al. (2021). Mechanism and function of root circumnutation. *Proceedings of the National Academy of Sciences*, 118(8). Doi:10.1073/pnas.2018940118
- Telewski, F. W. (2012). Is windswept tree growth negative thigmotropism?. *Plant Science*, 184, 20-28. Doi:10.1016/j.plantsci.2011.12.001
- Thompson, E. (2007). *Mind in life: biology, phenomenology, and the sciences of mind*. London, Harvard University Press
- Thompson, E. (2016). *Introduction to the revised edition*. In Varela F., Thompson E., & Rosch E. (Eds.) *The Embodied Mind: Cognitive Science and Human Experience* (pp. 17-23). MIT Press.
- Torrey, J. G. (1976). Root hormones and plant growth. *Annual Review of Plant Physiology*, 27(1), 435-459. Doi:10.1146/annurev.pp.27.060176.002251
- Toyota, M., & Gilroy, S. (2013). Gravitropism and mechanical signaling in plants. *American Journal of Botany*, 100(1), 111-125. Doi:10.3732/ajb.1200408
- Trewavas, A. (2005). Green plants as intelligent organisms. *Trends in Plant Science*, 10(9), 413-419. Doi:10.1016/j.tplants.2005.07.005
- Trewavas, A. (2007). Response to Alpi et al.: Plant neurobiology—all metaphors have value. *Trends in Plant Science*, 12(6), 231-233. Doi:10.1016/j.tplants.2007.04.006

- Trewavas, A. (2009). What is plant behaviour?. *Plant, Cell & Environment*, 32(6), 606-616. Doi:10.1111/j.1365-3040.2009.01929.x
- Trewavas, A. (2014). *Plant behaviour and intelligence*. OUP Oxford.
- Trewavas, A. (2016). Intelligence, cognition, and language of green plants. *Frontiers in Psychology*, 7, 588. Doi:10.3389/fpsyg.2016.00588
- Trewavas, A. (2017). The foundations of plant intelligence. *Interface Focus*, 7(3), 20160098. Doi:10.1098/rsfs.2016.0098
- Tronchet, A. (1945). Le comportement des vrilles en présence de tuteurs. *Bulletin de la Société Botanique de France*, 92, 147–152.
- Tronchet, A. (1946). Suite de nos observations sur le comportement des vrilles en présence de tuteurs. *Bulletin de la Société Botanique de France*, 93, 13–18.
- Tronchet, A. (1977). *La Sensibilité des Plantes*. Masson
- Turner, N. C. (1986). Adaptation to water deficits: a changing perspective. *Functional Plant Biology*, 13(1), 175-190. Doi:10.1071/PP9860175
- Tyree, M. T., & Sperry, J. S. (1989). Characterization and propagation of acoustic emission signals in woody plants: towards an improved acoustic emission counter. *Plant, Cell & Environment*, 12(4), 371-382. Doi:10.1111/j.1365-3040.1989.tb01953.x
- Ullman, S. (1980). Against direct perception. *Behavioral and Brain Sciences*, 3(3), 373-381. Doi:10.1017/S0140525X0000546X
- Vadillo, M. A., Konstantinidis, E., & Shanks, D. R. (2016). Underpowered samples, false negatives, and unconscious learning. *Psychonomic Bulletin & Review*, 23(1), 87–102. Doi:10.3758/s13423-015-0892-6
- van Bel, A. J., Furch, A. C., Will, T., Buxa, S. V., Musetti, R., & Hafke, J. B. (2014). Spread the news: systemic dissemination and local impact of Ca²⁺ signals along

- the phloem pathway. *Journal of Experimental Botany*, 65(7), 1761-1787.
- Doi:10.1093/jxb/ert425
- van Loon, J. J., de Boer, J. G., & Dicke, M. (2000). Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata*, 97(2), 219-227. Doi:10.1046/j.1570-7458.2000.00733.x
- van Overbeek, J. (1939). Phototropism. *The Botanical Review*, 5(12), 655-681.
- Doi:10.1007/BF02871651
- van West, P. V., Morris, B. M., Reid, B., Appiah, A. A., Osborne, M. C., Campbell, T. A., et al. (2002). Oomycete plant pathogens use electric fields to target roots. *Molecular Plant-Microbe Interactions*, 15(8), 790-798.
- Doi:10.1094/MPMI.2002.15.8.790
- Vanstraelen, M., & Benková, E. (2012). Hormonal interactions in the regulation of plant development. *Annual Review of Cell and Developmental Biology*, 28, 463-487.
- Doi:10.1146/annurev-cellbio-101011-155741
- Varela, F. J. (1992). *Autopoiesis and a biology of intentionality*. In Proceedings of the workshop "Autopoiesis and Perception" (pp. 4-14). Dublin City University..
- Varela, F. J., Thompson, E. & Rosch, E. (1991). *The embodied mind: Cognitive science and human experience*. London, MIT Press.
- Vidal, E. A., Arous, V., Lu, C., Parry, G., Green, P. J., Coruzzi, G. M., & Gutiérrez, R. A. (2010). Nitrate-responsive miR393/AFB3 regulatory module controls root system architecture in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, 107(9), 4477-4482. Doi:10.1073/pnas.0909571107
- Vivaldo, G., Masi, E., Taiti, C., Caldarelli, G., & Mancuso, S. (2017). The network of plants volatile organic compounds. *Scientific Reports*, 7(1), 1-18.
- Doi:10.1038/s41598-017-10975-x

- Volkov, A. G. & Brown, C. L. (2006). *Electrochemistry of plant life*. In: Volkov, A. G. (Ed.). *Plant Electrophysiology*. Springer, Berlin, Heidelberg. Doi:10.1007/978-3-540-37843-3_19
- Volkov, A. G., Adesina, T., & Jovanov, E. (2007). Closing of Venus flytrap by electrical stimulation of motor cells. *Plant Signaling & Behavior*, 2(3), 139-145.
Doi:10.4161/psb.2.3.4217
- Von Mohl, H. (1827). *Ueber den bau und das winden der ranken und schlingpflanzen*.
Heinrich Laupp.
- Wang, Q., Guerra, S., Ceccarini, F., Bonato, B., & Castiello, U. (2021). Sowing the seeds of intentionality: Motor intentions in plants. *Plant Signaling & Behavior*, 1949818.
Doi:10.1080/15592324.2021.1949818
- Weidenhamer, J. D. (2016). Plant olfaction: using analytical chemistry to elucidate mechanisms of plant growth and interaction. *Plant and Soil*, 407(1), 275-278.
Doi:10.1007/s11104-016-2998-7
- Weiler, E. W., Albrecht, T., Groth, B., Xia, Z. -Q., Luxem, M., Liß, H., et al. (1993). Evidence for the involvement of jasmonates and their octadecanoid precursors in the tendril coiling response of *Bryonia dioica*. *Phytochemistry*, 32, 591–600.
Doi:10.1016/S0031-9422(00)95142-2
- Weir, T. L., Park, S. W., & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology*, 7(4), 472-479. Doi:10.1016/j.pbi.2004.05.007
- Willson, M. F., & Traveset, A. (2000). *The ecology of seed dispersal*. In Fenner, M. (Ed.) *Seeds: the ecology of regeneration in plant communities*. (pp. 85-110). CABI publishing.

- Wilson, B. F. (1967). Root growth around barriers. *Botanical Gazette*, 128(2), 79-82.
Doi:10.1086/336383
- Wilson, M. (2002). Six views of embodied cognition. *Psychonomic Bulletin & Review*, 9(4), 625-636. Doi:10.3758/BF03196322
- Yates, F. E. (1983). *What is self-organization*. Princeton University Press
- Yates, F. E. (2012). *Self-organizing systems: The emergence of order*. Springer Science & Business Media.
- Yoshihara, T., & Iino, M. (2005). Circumnutation of rice coleoptiles: its occurrence regulation by phytochrome, and relationship with gravitropism. *Plant, Cell & Environment*, 28(2), 134-46. Doi:10.1111/j.1365-3040.2004.01249.x
- Yoshihara, T., & Iino, M. (2006). Circumnutation of rice coleoptiles: its relationships with gravitropism and absence in lazy mutants. *Plant, Cell & Environment*, 29(5), 778-792. Doi:10.1111/j.1365-3040.2005.01444.x
- Zhang, Y., & Friml, J. (2020). Auxin guides roots to avoid obstacles during gravitropic growth. *The New Phytologist*, 225(3), 1049. Doi:10.1111/nph.16203
- Zimmermann, M. R., Maischak, H., Mithofer, A., Boland, W., & Felle, H. H. (2009). System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. *Plant Physiology*, 149(3), 1593-1600.
Doi:10.1104/pp.108.133884
- Zimmermann, M. R., Mithöfer, A., Will, T., Felle, H. H., & Furch, A. C. (2016). Herbivore-triggered electrophysiological reactions: Candidates for systemic signals in higher plants and the challenge of their identification. *Plant Physiology*, 170(4), 2407-2419. Doi:10.1104/pp.15.01736

APPENDIX



The software SPROUTS has been designed and developed for the analysis of plants movement in different environmental conditions. The software was developed by using Python 3.7 to enable a user-friendly tracking for the considered key landmarks. The software was designed to work for any kind of growing setup that provides image streams from two cameras.

The software led the users to:

1. Perform a semi-automatic tracking of custom number of key points. At the beginning, the user is asked to identify the points to be tracked on the first image of both left and right cameras, then a tracking algorithm estimates the position of the points on the following frames. The user can check for position estimation errors eventually introduced by the automatic tracking procedure, re-mark the point to be tracked on the first available image, supervise the tracking process, and eventually adjust the trajectory of the point being tracked. At the end of the tracking process, the user can review the position of the tracked points in all the images and eventually correct the positioning errors manually. Such semi-automatic tracking is implemented using the Lucas Kanade computer vision method (Lucas & Kanade, 1981) based on optical flow, using a size of the search window of 10 by 10 pixels, a maximal pyramid level number (iterative lowering of image resolution) equal to 20 and the termination criteria of 30 maximum iterations.
2. Compute the 3D trajectories of each tracked point in real world dimensions. Three-dimensional trajectories are obtained from 2D trajectories acquired for both the

left- and right-side cameras using the Matlab triangulating function (Hartley, Zisserman & Faugera, 2004).

3. Save the 3D coordinates of the point in each frame as .cvs files.

The 2D and 3D positions and kinematic features are calculated from coordinates of landmarks with a dedicated procedure. The extraction module was developed in Matlab 2020a and was designed to process the 3D trajectory files obtained from the Video Processing steps described above (*see* Chapter 3). The 3D point position reconstruction algorithms provide the x, y and z coordinates of each point of the trajectory in a reference system with its origin on the lower left corner of the calibration chessboard. Since the positioning of the chessboard cannot be consistent on all the experiments, to enable the extraction computation of the correct features, the points' reference system is roto-translated to the plant's reference system (Fig. A.1.).

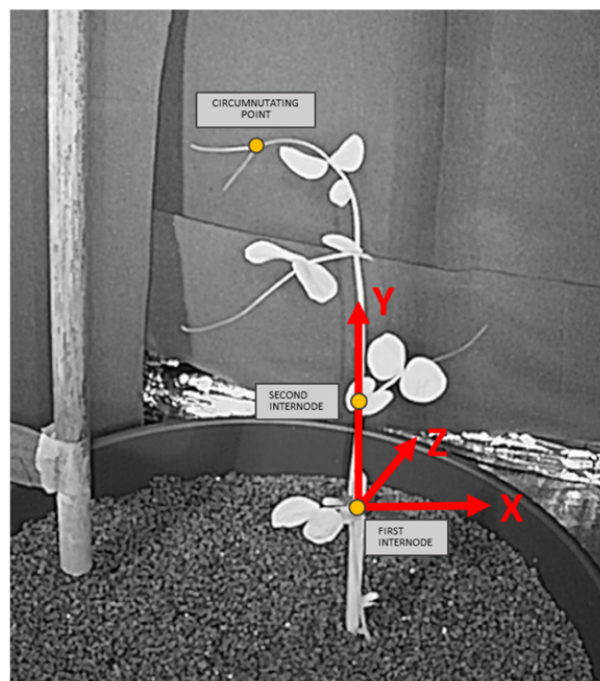


Fig. A.1. Photograph showing the plant reference system in which the y-axis corresponds to the plant's vertical axis, while the x and z axes lay on the plane perpendicular to the y-axis containing the system origin. (from Simonetti et al., 2021).

The new reference system is built such that the system origin coincides with one of the points along the vertical axis of the plant not showing relevant movement during the acquisition. The y-axis of the new 3D system corresponds to the plant's vertical axis, while the x and z axes lay on the plane perpendicular to the y-axis containing the system origin. To correctly identify the plant's vertical axis and origin, the user is required to position two points along the plant's axis during the video processing. The vertical axis is the line passing through these two points. In the case of *P. sativum* plants, the y-axis was set as the line passing through the first and second internode, with the system origin being the first internode (Fig. A.1.). Once the reference system is aligned to the plant's system, the software detects circumnutating movements. Taking the X–Z components of the 3D movement, the software computes the angle (α) between the horizontal axis (x-axis) and the movement vector of each frame, considered as the vector between the tracked point at frame t (P2) and the same point at frame t-1 (P1). A single *circumnutation* movement is considered as the movement occurring between two local maxima of the α angle. After the segmentation of the *circumnutation*, a set of features aimed at describing the movement of plants is computed. The features extracted by the SPROUT software can be classified in four main categories such as:

1. Circumnutation related features, which provide a quantitative description of each circumnutation extracted from the movement segmentation such as (i) duration (i.e., seconds needed to complete the whole circumnutation movement); (ii) mean/max/min circumnutation speed (i.e., values for mean, maximum and minimum for the speed traveled by the circumnutating point along each circumnutation); (iii) circumnutation path length (i.e., the overall 3D path travelled by the circumnutating point, which is computed as the sum of all the Euclidean distances between subsequent point positions during the single circumnutation).

(iv) circumnutation center (i.e., geometric center of gravity in the X–Z plane computed as the mean of each coordinate for all the points constituting the circumnutation); (v) circumnutation center distance from plant origin (i.e., euclidean distance between the circumnutation center and the plant origin in the X–Z plane); (vi) circumnutation center speed (i.e., speed of the circumnutation center computed as the distance traveled by the circumnutation center in two consecutive frames on the X–Z plane divided by the time between frames); (vii) circumnutation centroid (i.e., considering the points of the trajectory described by the circumnutation on the X–Z plane, the algorithm identifies the region of interest as the closed line obtained by linking such points. The pixels in the polygon area are set to 1, while the others are set to 0); (viii) the circumnutation centroid (i.e., the geometric center of gravity in the X–Z plane computed as the mean of each coordinate for all the points with value 1 contained in the circumnutation area); (ix) circumnutation centroid distance from plant origin (i.e., the Euclidean distance between the circumnutation centroid and the plant origin in the X–Z plane); (x) circumnutation centroid speed (i.e., speed of the circumnutation centroid computed as the distance traveled by the circumnutation centroid in two consecutive frames on the X–Z plane divided by the time between frames); (xi) circumnutation main axis (i.e., the principal axis of the ellipsoid of the circumnutation, computed as the maximum distance between two points of the circumnutation trajectory in the X–Z plane); (xii) circumnutation area (i.e., the sum of pixels with a value equal to 1 obtained from the binarization of the circumnutation trajectory on the X–Z plane as described for the calculation of circumnutation centroid); (xiii) Direction (i.e., clockwise or counterclockwise direction of the circumnutation. For each circumnutation, the software computes the sum of all the angles between the

movement vector at time t and the movement vector at time $t+1$. The direction, then, is determined according to the following logic: if the resulting sum is equal to $2\pi \pm 1.2$, then the direction is counterclockwise, or else if the resulting sum is equal to $-2\pi \pm 1.2$, then the direction is clockwise. For all other cases, no direction is assigned).

2. Point related features, which are metrics related to circumnutating point kinematics along the movement such as: (i) mean and maximum speed (i.e., mean and maximum speed of the point reached along with the whole movement. Speed is computed as the distance traveled by the point between consecutive frames divided by the time between frames); time of maximum speed (the time at which maximum speed is reached both as absolute time and as a percentage with respect to the whole movement).
3. Experimental specific features such as: (i) center/centroid distance from stimulus (i.e., the Euclidean distance between circumnutation center/centroid and the stimulus in the X–Z plane); (ii) the angle between circumnutation main axis and plant stimulus axis (i.e., the angle between circumnutation main axis and plant-stimulus axis computed as the line passing through the plant origin and the stimulus on the X–Z axis); (iii) the minimum distance from stimulus (i.e., the 3D Euclidean distance between the stimulus and its closest point of the circumnutation trajectory).