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CHICKPEA PLANT IT'S MOLECULAR REGULATION OF ROOT HAIRS: A REVIEW

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ABSTRACT

Chickpea is one of the most important leguminous, food crop. Conventional breeding has resulted in several important improvements in this crop. Chickpea is an important part of human nutrition as it has good nutritional value. This results suggest that a collective effect of superior morphological and physiological root traits confers better nutrition of chickpea genotypes in low nutrient soil conservation. We investigated the morphological (root length, root hairs) and physiological (root exudation of protons and phosphatase enzymes) root traits and related them to uptake of twelve nutrients by ten selected breeding lines of chickpea. Reactive oxygen species (ROS) acts as a signaling molecule that plays a very crucial role in maintaining normal plant growth. Salinity causes loss of chickpea crop in various regions and salt accumulates Na+ and Cl- ions in the tissues of plant that causes detrimental effects to chickpea. The increase of ROS can be decreased by the addition of Nod Factors (NFs) in the root hair cells. The level of calcium ions increases that lead to increase in the ROS species accumulated at the top of root hair cells.
***Keywords*:** Chickpea, molecular regulation, root hairs

Introduction

Cultivated chickpea also known as *Cicer arietinum L* is a self-pollinating and diploid crop. It grows annually during the colder seasons of the year when temperatures are between 70° to 80°F during the day and 64° to 70°F during the night and grows best on fertile loam and sandy soil. It ranks third among the most important legume found in this world.

Chickpea has been classified as follows:

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Faboideae
Genus	:	*Cicer*
Species	:	*C. arietinum* (Wood *et al*, 2011)

The largest part in the seed is the embryo, which consists of two cotyledons which are joined at their axial surfaces, a small hypocotyl and a radicle located in the chickpea beak. The embryo is surrounded by a seed coat which acts as its protective covering. It also has a tap root system which enhances its capacity to withstand conditions such as drought.

Chickpea is the cultured legume, *Cicer arietinum*. It is the refined pulse from the tribe Cicereae (Family Fabaceae). Other names for this crop of Neolithic southwest Asian sources comprise garbanzo beans in the Americas, hamaz in Arabic countries, nohud or lablabi in Turkey, shimbra in Ethiopia, and bengal gram or chana in India. Chickpea ranks third in the world's invention of seed legumes, after soybean and pea. (Fuller *et al*, 2006)

The morphology and shape of desi seeds and kabuli chickpea (*Cicer arietinum L.)* genotypes were studied using mild microscopy with spots of protein, starch, β-glucans, compounds. Kabuli seeds had a skinny seed coat due to the skinny layers of the palisade and the parenchyma which contained small pectic polysaccharides and small proteins. The outer layer of the palisade is different in dimension from one to two cells, resulting in a mixed and occasionally colored appearance of the seed surface. On the contrary, the layers of the desi palisade have been company and very thick. Hourglass cells were comparable in each seed varieties, but no longer in a clear desi line which had extraordinary cells. The inside of the seed coat contained both pectic and proteinaceous substances. Cotyledon consist of a single outer epidermal layer of starch-free protein cells, with thick outer phone walls; the dimension and form are one of a kind from the abaxial and adaxial faces. The sub-epidermal cells on the abaxial surface were comparable to epidermal cells. These findings assist to explain the differences in functional conduct between giant sorts of chickpea seeds (Wood *et al*., 2011)

Chickpea plants has 8 strong taproot system with 3 or 4 rows of lateral roots Theparenchymatous tissues of the roots are rich in starch. All the peripheral tissues disappear attime of maturity and are substituted by a layer of cork. The roots grow 1.5- 2 m deep. Chickpea roots bear Rhizobium nodules. They ate of the carotenoid type, branched with laterally flattened ramifications, sometimes forming a fanlike lobe. Chickpea is a cool annual crop that thrives at 70 ° to 80 ° F during daytime temperatures and at night temperatures of 64 ° to 70 ° F. They produce a good yield in dry conditions due to their deep tap roots. Heavy rains (more than 30 per year) show reduced yields due to outbreaks of disease and sleeping problems due to overgrowth of vegetables. Areas with easy, well-distributed rainforests produce high yields and quality chickpea seeds. Chickpea thrives in well-drained, fertile sandy loam with a fine interior. Good flow is necessary because even short periods of flooded or flooded areas slow down growth and increase the chances of root rot. Good quality certified chickpea seeds should be used regularly. These seeds should be high in germination percentage (over 85%), without damage, and without weed seeds. (Croser *et al*., 2003)

Root hair patterning and development

Cicer arietinum is a short(less than a metre) annual herb. It assumes the growth habitdepending on the angle of the branches and the surface of oil. It can be 'erect, spreading, semi-erect, semi-spreading and prostrate. Plant has the bushy appearance as the branching starts from the ground level. Almost whole of the plant surface is covered with glandular andnon-glandular hairs. The former secretes malic, citric and oxalic acid acting as a defence mechanism against sucking pests. Solubilising of the soil nutrients is done from the exudation of roots. (Varshney *et al*., 2017)

The root system of the chickpea plant is robust as it is characterized by a thick tap root alongwith several developing side roots. The endodermis is thin, exodermis is absent and theepidermis is hairy. (Varshney *et al*, 2017) Root hairs being highly specialized cells play an important role in providing water and mineral nutrients to the plant. There is presence of nodules on roots leading to biological nitrogen fixation indicating the symbiotic relationship between the chickpea and Rhizobium bacteria (Mesorhizobiumciceri). The plant survives in the moisture stress conditions because the tap root system is robust enough that it reaches more than 3m in soil.

The Chickpea plant is considered to be poor in nutrition and drought prone, without the fertilizers and irrigation. In order to overcome the abiotic stress and to enhance the productivity of the chickpea plant favourable root traits, which capture the nutrients and water from soil play a vital role. Root traits, be it morphological which include root hairs and root length and physiological which includes exudation of protons and enzymes facilitate the use of fertilised nutrients in soil efficiently. (Gahoonia *et al.*, 2007)

Such plants when grown in weathered or highly alkaline soil experience phosphorus stress.So for the maximum yield application of mineral phosphorus fertilizers are required. (Ghiath Alloush, 2003)Normally Chickpea when cultivated in a field is nodulated and fixes the nitrogen leading to acidification of rhizosphere which in turn increases the phosphorus availability in soil (Ghiath Alloush, 2003)

Chickpea has fewer root hairs with restricted root system consisting primarily of main apex and branching roots from the lateral roots. Under low phosphorus supply total lengths and root mass is slightly reduced. Also morphological changes occur under phosphorus stress conditions. In many ways the plant responds to phosphorus stress like it reduces the alkalinisation /acidification of rhizosphere, modifies the root morphology and accumulation of organic acids in tissues. (Ghiath Alloush, 2003)

Molecular mechanism of root hair cell formation

Plant science is a vast branch to study and a complex field to understand and to carry on research for the sake of knowledge. Roots are still one of the most challenging subjects to study, but its importance is unquestionable. The main functions of plant root systems are Anchorage, support, and water and nutrient uptake, which carry forward many mechanisms that are important for a healthy growth and development of plant. (Carvalho and Foulkes, 2018)

A root system of any plant is geotropically positive and achlorophyllous which includes primary root. Lateral roots, the apical meristem, a root cap and a root hair. Root hair is a cylindrical lateral extensions of root epidermal cells present on the tips of lateral roots which are even visible with naked eyes and light microscope. There are three zones in root system, Meristematic zone, Elongation zone and Maturation zone, root hair are found only in zone of maturation. A root hair cell consists of cell wall, cell membrane, vacuole, cytoplasm, nucleus. The main functions are to absorb water (osmosis) and nutrient molecules (active transport). It augments the total surface area of a root system, dramatically increasing its absorption capacity

The molecular mechanisms exhibited by the root hair of the chickpea plants are osmosis of water molecules, Uptake of Magnesium molecules through active transport mechanism, nitrogen fixation and so on discussed in later. Studies and experiments on the chickpea root traits will support breeding of nutrient-efficient and drought-tolerant high-yielding varieties for marginal and dry areas. Exploration of genetic diversity in root traits of chickpea germplasm will be beneficial for breeding of varieties able to withstand nutrient and water stresses.

Relationship between nutrients and root hair formation:

The chickpea plant grows on nutrient-poor and dry soils; hence the role of root traits in capturing soil resources may be central for their economical outputs. The 15 mineral elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo, Na, Cl, Co) essential for the higher plants are mainly absorbed by roots from soil (Marschner, 1995). Experiment of Gahoonia *et al*, shows physiological (root exudation of protons and phosphatase enzymes) root traits and related them to the uptake of twelve nutrients by ten selected varieties/breeding lines of chickpea and the results said that the low nutrient soil environment is favourable for the morphological and physiological root traits of the chickpea plant. The nutrients acquired and accumulated are Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Boron (B), Molybdenum (Mo). The genotypes inducing greater rhizosphere acidification and having prolific root hairs absorbed relatively higher amounts of the nutrients (K, P, Fe, Mn and Zn) whose availability in soils is usually low, uptake of such nutrients is through the active transport molecular mechanism.

Formation and characteristics the root hairs such as volume, length, longevity depends on n=both genetic and environmental factors. The environmental factors that affect the formation of root hairs are soil pH and soil calcium concentration (Ewens and Leigh, 1985), the presence of bicarbonates (Bergmann *et al*, 1958), length and number is regulated by mineral nutrients, particularly Nitrate and phosphate. When the concentration of nitrate is low, high number of root hairs with long length are formed (Fohse and Jungk, 1983)

Phosphate in the soil has lower effective diffusion coefficient than potassium, hence the acquisition of Potassium is assumed to depend on root hairs. The rate of uptake of potassium per unit root length was related to volume of the root hair length, directly. The length of root hairs was negatively related to the phosphate concentration in plant tissue, hence limiting phosphate in contact with root epidermis regulates root hair formation (Jungk, 2001)

Previous studies have revealed that phytohormones such as auxin, ethylene, cytokinin, promote root hair growth while ABA and brassinosteroid inhibit root hair growth. (Grierson *et al*. 2014; Lee and Cho 2013) The process of Nodule development and nitrogen fixation by legumes require Cobalt (Co) which is very essential for the both the mechanisms. (Dilworth and Bisseling, 1984)

Chickpea association with Rhizobium and Root-Nodule Development Genes

Chickpea is a legume plant which form root nodules that support biological N-fixation and also host symbiotic N-fixing bacteria in which root hair plays an important role. (Ghanem *et al.*, 2012). Significant proportion of its N requirement of chickpea is fulfilled through symbiotic N fixation. Inoculation of chickpea with adequate number of rhizobia results in a significant increase in the number of nodules, nodule dry weight and N fixation (Sattar *et al.*, 1993). Growth of chickpea is dependent on nodulation by effective rhizobial strains. (Nitrogen (N) is one of a plant nutrient which is highly important for crop productivity. There is a need to find alternatives to N fertilisers 4 such as biological nitrogen fixation, a microbial process that converts atmospheric nitrogen into a plant-usable form. Continuous cultivation for cereal cropping has led to depletion of soil organic carbon and N in the major cereal growing areas. This has directly affected cereal crop yields and cereal grain protein concentrations and farmers are not able to obtain maximal yields for their crop. Although chickpea (*Cicer arietinum L.*) is di-nitrogen (N2) fixing pulse legume that has been widely grown widely and accepted in cereal rotations in the region, limited work has been conducted on management options to enhance its ability to fix atmospheric N. Recently, N fixation inputs and N balances, i.e. the difference between N fixation inputs and N in harvested products (outputs), as well as rotational benefits of chickpea have been quantified revealing its importance in nitrogen fixation. Chickpea roots hairs form nodules that help in the symbiotic association with nitrogen-fixing rhizobia. The expression of putative nodulation receptor kinase (LOC101507037) and calcium/ calmodulin-dependent serine/threonine-protein kinase DMI3 (LOC101513751) were down-regulated in the sensitive genotype both at Vegetative stage (VS) and Reproductive stage (RS). The genotype showed marginal down-regulation at RS only. Whereas, receptor-like kinases 3 (LOC101496137) and CLAVATA1 (LOC101488348) were up-regulated at VS and RS in the tolerant genotype. (Bhaskarla *et al*, 2020). Plant with root hairs exuded significantly more carbon into the soil than plants without hairs and extended the rhizosphere in radial and axial directions. The higher exudation and the increased rhizosphere extension might be an advantage for plants with root hairs because both favour plant–microbial interactions and therefore nutrient mobilization in the rhizosphere.

ROS mediated changes in root hair

Reactive oxygen species (ROS) acts as a signaling molecule that plays a very crucial role in maintaining normal plant growth. To improve the crop yield, the coordinated growth of plants is very much necessary. ROS is generated in the chloroplasts, mitochondria and peroxisomes of the plants. (Sharma *et al*, 2012) During the aerobic metabolism of plant, ROS is generated as a by- product. Under unfavourable conditions, plants released high amount of ROS species. The source of ROS is generally oxygen which is not much reactive in the plants. But the unreactive ROS is converted into reactive ROS by modification in cellular organelles. This will prevent ROS to perform their original functions in plants. They will release processes including pathogen defense, programmed cell death (PCD), and stomatal behaviour which will reverse the reactions and normal plant growth does not take place. (Huang *et al*, 2019)

ROS in plants are present mainly in two forms:-

- Ionic state- superoxide anions (O^{2-}), hydroxyl radical (OH-)
- Molecular state- hydrogen peroxide (H2O2), singlet oxygen (O_2) (Sharma *et al*, 2012)

ROS formation-

1. Generation of ROS is due to various environmental stresses like drought, salinity, chilling, metal toxicity, and UV-B radiation
2. Attack of pathogens will lead to disruption of cellular homeostasis that will increase ROS level in plants.
3. The electron transport activities inside various cellular organelles will lead to formation of ROS by inevitable leakage of electrons onto O2.
4. Due to generation of ROS through environmental stresses as discussed above, it will cause peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells. (Huang *et al*, 2019)

Salinity effect on chickpea and ROS changes-

Chickpea is grown in many arid and semi- arid areas of world due to its natural tendency of fixing atmospheric nitrogen, more nutritional availability. Various types of abiotic stresses occur in plants, but in these areas, salinity is much more common. Salinity causes loss of chickpea crop in that area. The stress of salt accumulates Na+ and Cl- ions in the tissues of plant that causes detrimental effects to chickpea. This will lead to disturbance in the osmotic environment in the plant. (Kaashyap *et al*, 2018)

Inside plants various sensory mechanisms occur that respond to the stress conditions prevailing in the environment. The first mechanism that occurs in plants is the sudden release of reactive oxygen species (ROS). ROS will lead to-

- Detoxification of hydrogen peroxide (H_2O_2) mediations.

- Activation of calcium (Ca^{2+}) mediations.
- Activation of plasma membrane containing calcium transporting ATPase that induces Ca^{2+} ions.

These calcium ions when released due to ROS mediated changes will lead to activation of protein kinase gene - mitogen activated protein kinases (MAPK) and calcium dependent protein kinases (CDPK). (Kaashyap *et al.*, 2018)

These protein kinases genes lead to phosphorylation and de-phosphorylation of signal cascade proteins. With the release of signals, peroxidases enzyme comes into action and hydrogen peroxide starts disintegrated.

ROS (reactive oxygen species) → Cationic Peroxidase → oxidation of cell polymers →nucleus → transcription of salt stress responsive genes (Kaashyap *et al*, 2018)

Nod factors in root cells and relation with ROS-

On the apical region of root hair cells ROS gets accumulated. As soon as the rhizobial infection takes place in legume plants like chickpea, the level of ROS increases. This increase of ROS can be decreased by the addition of Nod Factors (NFs) in the root hair cells. (Luis Cardenas and Carmen Quinto, 2008)

The exposure of NFs is done at various time intervals. In some the change was observed within minutes and in some the change was observed in seconds. The Ca^{2+} influx and ROS present at the tip of root shows a change when exposed to NF. The Ca^{2+} level increases within 15-60 seconds and the level of ROS is increased within 15-30 seconds.

Inside the nucleus, there is a spike increase in the level of Ca^{2+}. Within 10-15 minutes, gene expression can be observed in the root hair cells. ROS activates the calcium channels at the tip of root hair cell and this increases the level of Ca^{2+}. The increase of calcium ion influx directly leads to increase in NAD(P)H. (Luis Cardenas and Carmen Quinto, 2008)

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