International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 22-12-2020; Accepted: 12-01-2021: Published: 23-01-2021
www.botanyjournals.com
Volume 6; Issue 1; 2021; Page No. 242-247

Differential effects of N availability on growth and development of lateral roots

Vijendra Pal Singhal, SK Mehar*
Department of Botany, JNV University, Jodhpur, Rajasthan, India

Abstract

Due to the very low levels of water and soil nutrients desert ecosystems are difficult habitats to survive for plants. Under such conditions, survival strategies of desert plants essentially involve sensing the resources available in the surrounding, and modifying their development plans accordingly. Our study of the root system architecture of *Crotalaria medicaginea*, a plant of Indian desert, indicate the presence of some features which are modified according to the amount of nitrogen in surrounding.

We have investigated the growth of *Crotalaria* at seedling stage with variable levels of N in the growth medium (0, 5, 10, 25, 50, 75 and 100%), of the total complement in the media (Murashige and Skoog media). The number and lengths of first order lateral roots were higher in the middle range of N supply and lower in the other treatments. Basal Zone and Apical Zone Lengths were also significantly influenced by the amount of N available. The Basal Zone Length was very low in treatments with more N, indicating the origin of first order laterals near the base of the main root, and this growth strategy was responsible for the increased length of the Apical Zone Length in the similar treatments, indicating a longer tip of the main root without lateral roots when roots experienced excess supply of nitrogen. Basal and Apical Zone Length had significant negative relationship (r^2=0.05, $p<0.001$).

Keywords: root system architecture, first order lateral roots, basal zone length, apical zone length

Introduction

Invasion and colonization of the terrestrial habitats by plants was dependent on the development, and successive evolution of the root system. Plant roots served the purpose of anchoring the plants and acquisition of water and essential nutrients from soil. While taking up water and nutrients from soil, roots have to forage for the resources which are not homogenously distributed. It has been observed that those plants which have a proliferating and dynamically responding root system gain competitive advantage in the plant community (Robinson *et al.*, 1999) [27]. The root system has to function very efficiently and in cost effective manner, because the productivity of the plant depends on the nutrients which are taken up by the roots, which are further invested in the above-ground biomass to facilitate carbon assimilation (Evans and Terashima, 1988) [7]. The three dimensional structural configuration of the root system forms the Root System Architecture (RSA). The RSA has to, and can change drastically in response to changes in nutrient levels in the soil environment, particularly for the essential macronutrient nitrogen (Robinson, 1994; Lynch, 2013; White *et al.*, 2013; Giehl *et al.*, 2014) [26, 12, 19, 30, 11].

Differential availability of N has been reported to modify the developmental planning of the root system and relative growth rates of different components of the RSA. For example, presence of nitrate in relatively higher quantities can inhibit the growth of the roots, specifically the elongation rate of lateral roots, once they emerge from the primary roots (Zhang *et al.*, 1999) [33]. This morphological change is governed by the demand of N for the plant, and is influenced by relative availability of other resources and growth phase of the plant. Not only the excess of N could limit the growth of roots, but the limited N in soil environment could also inhibit the root growth when plants are grown under N limited condition for prolonged period. It has been argued that limiting the growth of roots in N limited condition could be preventive mechanism to avoid the root's extension into soil zones with poor returns of N on the investments vested in root growth (Araya *et al.*, 2014) [1]. The developmental patterns of primary and lateral roots may differ for the similar external supply of nutrients, for example, it has been observed that lateral roots continued to elongate for foraging N when they received adequate amount of nitrate (Remans *et al.*, 2006) [24]. This probably reflects the choice in decision to promote a particular RSA component over other based on cost benefit issues. Similar to nitrate, ammonium has been shown to inhibit the growth of primary root (Liu *et al.*, 2013) [17], but as observed by Lima *et al.* (2010) [15], locally stimulate the branching in lateral roots. This results in the formation of profusely branched lateral roots. Therefore, these results suggest that development and growth of plant roots is quite variable depending on the amount of nutrients (N) available in the external environment, and could also be influenced by the spatiotemporal supply or limits to the N resources. Structure and function of the primary and lateral roots are quite comparable, and both are essentially required for the uptake of water and nutrients in plants. After origin, lateral roots have the capacity to initiate further roots, which leads to higher order lateral roots. Metabolic cost of the development of lateral roots are comparatively lesser than the primary roots, hence, they influence the overall architecture of the root system, and are differentially promoted to explore and acquire nutrients, especially when they are in limited availability. For example, under the deficiency of N, there are evidences of increase in the length of lateral roots (Pan *et al.*, 1985) [23].

Deserts are one of the most inhospitable habitats of the world, where the environment is adverse to the extremes. The severity has been variously defined and elaborated; for example, it is referred to as “a place that was abandoned”, by the word ‘tesert’ in the ancient Egyptian hieroglyph. The local dialect in Rajasthan mention desert as “Marwar”, which has the origin in Sanskrit word “Maru-desa”, meaning the “land of death”. Such habitats definitely can only be inhabited by organisms specially adapted to the conditions. The Thar desert of India, despite the harsh conditions has over the course of time become home to a large number of plants and animals, which are uniquely adapted to the prevailing conditions of the land. The plants have become adapted, not only the extremes of temperature and scarce availability of water, but also to the limited availability of essential nutrients like N. Plants have to modify their developmental programming and alter the phenotype to extract the essential resources from soil as and when they become available, dependent on several other abiotic, and biotic factors in the desert. Developing better understanding of the growth and development of root system of such desert plants under differential availability of essential resources will enable us to better appreciate the development of plant communities and functioning of the desert ecosystem.

Crotalaria L. (Fabaceae) is one of the largest genera of Papilionoideae consisting of about 700 species. The species is extensively spread in different continents, mostly in the tropical and subtropical regions. One of the species *Crotalaria medicaginea* is native to the Indian Thar desert. It is an erect and branched annual herb, and widely distributed in the Thar desert (Bhandari, 1978) [3]. Due to the presence of alkaloids, tannins, and saponins in good quantity, this is medicinally and economically important plant.

In the present study we have made an attempt to investigate the change in relative growth rate of lateral roots by subjecting the plants to varying amounts of N in MS nutrient media. Understanding the response of *Crotalaria* to differential availability of N will enable us to better appreciate the response of such desert plants to limited availability of essential nutrients, and the approach resorted by them to cope with such situation.

Materials and Methods

Seeds of *Crotalaria* were collected from naturally growing plants in Jodhpur, Rajasthan, India (26.350932N, 73.046042E). Although the seeds were viable, they failed to germinate when placed in growth media. To establish the seedlings for the growth study, the seeds were subjected to acid treatment, following which they were washed repeatedly in distilled water. The seeds so treated were transferred to half strength Murashige and Skoog media (Murashige and Skoog, 1962) [22], with different levels of N (0%,5%,10%,25%,50%,75% and 100%, relative to the full complement of N in the MS media), to compare across wide range of N levels. These were than kept in growth room, illuminated with cool fluorescent tubes (14.4 W m^{-2}) at 28 ±1°C. During the entire period of growth of the seedlings, they were photographed every day at constant angle to observe the growth of roots (main and lateral roots). The digital images were analyzed with image J. For enumeration of different root system architectural parameters, the approaches used by (Chevalier *et al.*, 2003) [4] and (Kellermeier *et al.*, 2014) [14] were mainly referred. Statistical analysis was performed with IBM SPSS.

Results and discussion

Root growth in terms of increase in length and the branching are iterative processes. Differences in these attributes are responsible for change in morphology of the root system (Fitter, 1987) [9]. Regular observation of the growth of roots under different treatments enabled us to follow the transition on daily basis, as well as we could compare the features across certain specified intervals of time to develop insights of major shifts in growth patterns.

Lateral roots of first order started to develop from 6th day and gradually the number of lateral roots increased, so was the length of the laterals. Maximum increase on 15th days as compared to 10th day was observed in 25 and 50% treatments (upto 31%). The change in number of lateral roots and the gradual increase in their lengths are displayed in Figures 1 and 2. A two-way ANOVA was conducted for comparing the effects of number of days and the levels of N treatment (independent variables [IV]) as well as their interaction on the number and length of first order lateral roots (dependent variable [DV]). Effects of number of days and N levels on the number of first order lateral roots, and their lengths were statistically significant ($p<0.05$ and $p<0.001$ for the number; and $p<0.001$ for the length), however, their interaction was not (Table 1). With the increase in the number of first order lateral roots, their length constantly increased and it was linearly related to the number of first order roots ($r^2 = 0.722$, $p<0.001$), the linear relationship is being shown in the figure 3.

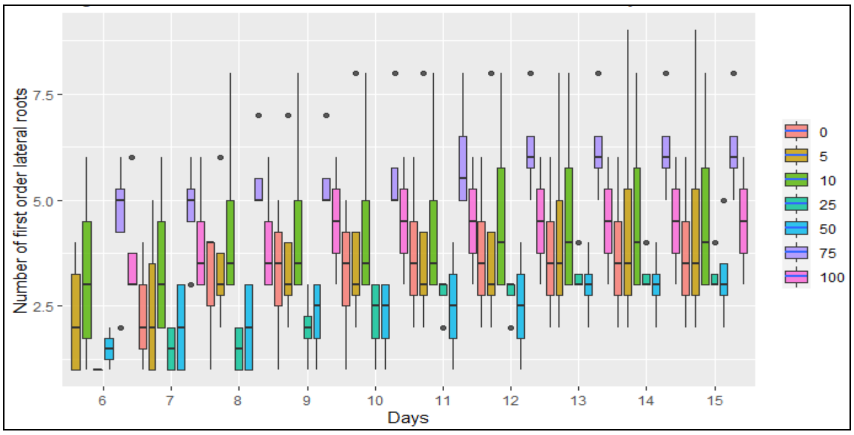

Fig 1: Number of first order lateral roots on different days

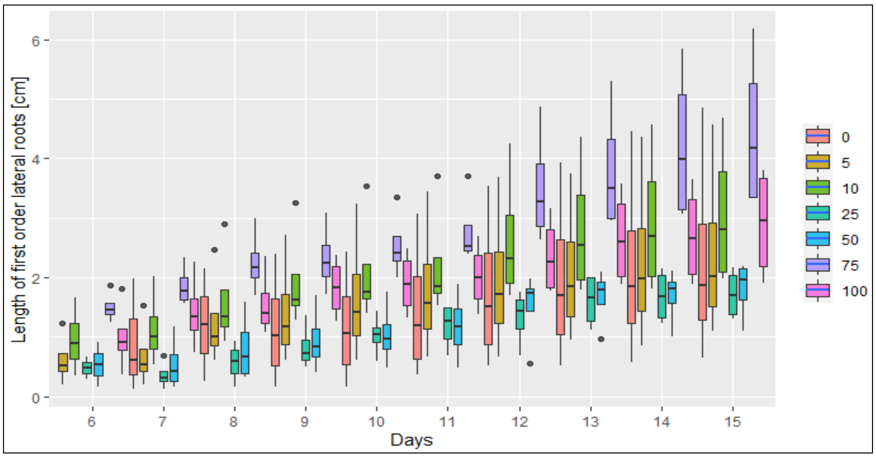

Fig 2: Length of first order lateral roots on different days

Table 1: Analysis of variance of effect of number of days and N treatment on number and length of first order lateral roots

	Number of first order laterals				**Total length of first order laterals**		
	Sum Sq	Mean Sq	F value	Pr(>F)	Mean Sq	F value	Pr(>F)
Days	58.606	6.512	2.307	0.017*	10.124	12.744	0***
N levels	326.773	54.462	19.293	0***	16.686	21.003	0***
Days : N levels	13.190	0.249	0.088	1.000	0.163	0.205	1

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

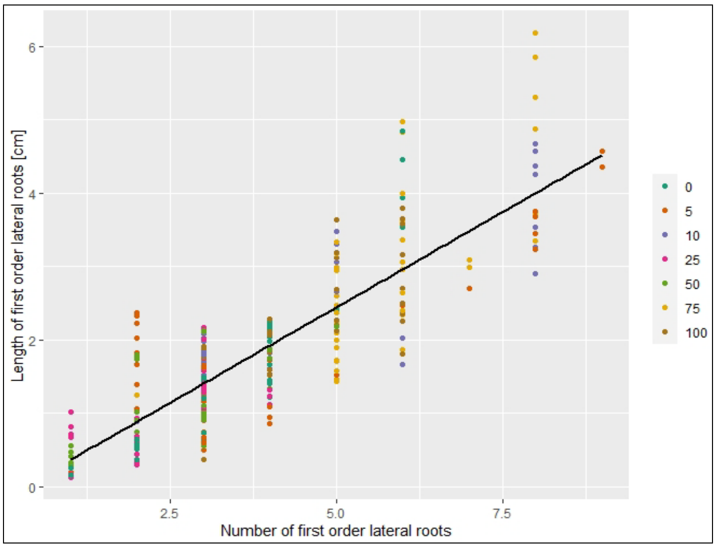

Fig 3: Relationship between number of first order lateral roots and length

Basal zone length represents the minimum distance from the junction of root-hypocotyl to the first lateral root origin. The treatments with higher N were having the basal zone length reduced in comparison to the treatments with lower levels of N. The middle levels of N viz., 10 to 50% had almost similar basal zone lengths amongst them, and the least value was recorded in 100% N treatments on 15th day (Figure 4). Analysis of variance results revealed that only the N levels resulted in significant variation in the basal zone length. In contrary to this, Apical Zone Length, which represents the length of the main root post the last lateral root, displayed the reverse trend. The Apical Zone Length was comparatively higher in treatments with greater N levels (except for 10% N; Figure 5), and similar to the Basal Zone, only the N level factor caused significant variation in apical zone length, the number of days and the interaction between days and treatments being non-significant (as with Basal Zone Length, Table 2.). Basal and Apical Zone Lengths were negatively related ($r^2 = 0.05$, $p<0.001$).

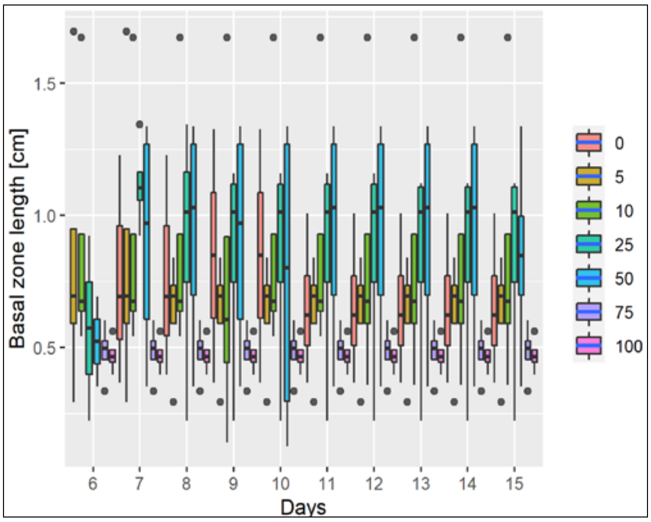

Fig 4: Basal zone length

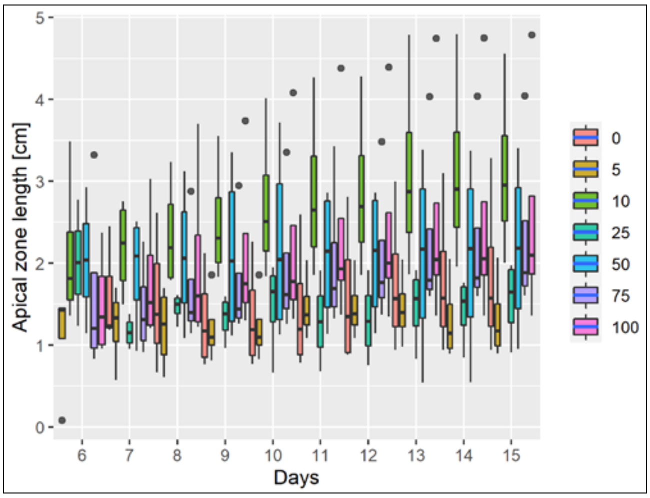

Fig 5: Apical zone length

Table 2: Analysis of variance of effect of number of days and N treatment on Basal and Apical Zone Length

	Basal Zone Length				Apical Zone Length		
	Sum Sq	**Mean Sq**	**F value**	**Pr(>F)**	**Mean Sq**	**F value**	**Pr(>F)**
Days	0.264	0.029	0.219	0.992	0.870	1.045	0.406
N levels	7.263	1.210	9.014	0***	9.847	11.823	0***
Days : N levels	1.200	0.023	0.169	1.000	0.145	0.174	1.000

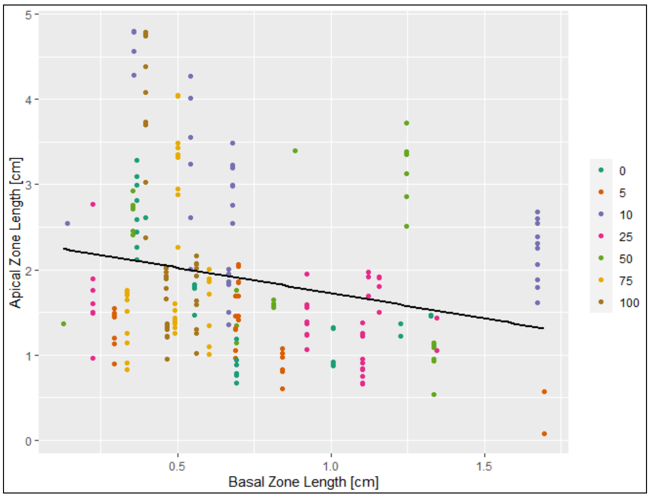

Fig 6: Relationship between Basal Zone Length and Apical Zone Length

During the life of the plant, root system develops and extends into the soil. The resulting architecture of root system then determines the efficiency of nutrient acquisition from the surrounding (Garnett *et al*., 2009) [10]. Since the changes in morphological traits of plant parts are made on the basis of relative analysis of benefits in comparison to the cost of production, root diameters due to their relationship with the total biomass of the root, are related directly to the investments in the below ground growth plan of a plant (Eissenstat, 1992) [6]. The reason for this is, on approximate basis, the biomass of a segment of root will vary more or less in tune with its diameter squared. Based on this, the amount of investment a plant makes in terms of carbohydrates for the development of roots, root diameter will influence the maximum length and the number of roots in the system (Bidel *et al*., 2000) [2]. This has been attributed to be the reason that the root system architecture of many plant species comprises mainly of very fine roots. This optimizes the ratio of the length of root (for uptake) to the weight of root (the investment made). If this could be considered as a sound strategy, do all plants will work properly with very fine roots? The answer is probably a No, because, roots with very thin width have poor penetrating power in the soil (Materechera *et al*., 1992; Clark *et al*., 2008), besides, the roots have to develop required internal structures that are needed for the transport of water and nutrients (Jordan *et al*., 1993; Jaramillo *et al*., 2013) [13, 12]. These reasons are among the factors which shape the architecture of the root system, and modify it in response to the availability of resources in the vicinity of the roots.

We have recorded comparatively more and longer lateral roots under the moderate supply of N, this agrees with the studies mentioned above, because the developing lateral roots were able to take up N in cost effective manner in these treatments. At very low N levels, the development of primary roots was probably not so rewarding, because of accompanied increase in the buildup cost of the main root, besides, due the uniform distribution of N in the external medium, there was no gain in extending the roots. Under high N treatments, for the similar reason that the N was easily available to the shorter roots also, there was no need to extend the roots further in the medium. An increase in the length of lateral roots is found to increase the acquisition capacity for N (Linkohr *et al*., 2002; Wang *et al*., 2006) [16, 29], further, it has been reported that the fine roots have the potential to take up more nitrogen in comparison to the more thick roots on dry weight basis (Sullivan *et al*., 2000). Along with this one more fact needs to be taken care of, that is, the change in quantity of one nutrient will influence the requirement for other nutrients. In the confined uniform supply of the nutrient medium, this conditioning becomes important from the plant's perspective. This has been addressed in earlier study of Lynch *et al.* (2012) [18], where they have reported that the availability and distribution of N source has been shown to influence investment of C and the architecture of plant root system. Further, McCully (1995, 1999) [20, 21] and Rich and Watt (2013) [25], have demonstrated that root architecture is controlled by the internal development program of the plant, and the interaction between the plants physiological state and the external biotic and abiotic stimulus in the root environment.

Linkohr *et al.* (2002) [16] demonstrated changes in the nitrate greatly influence the lateral root length. Primary root length was found to decrease with increase in nitrate availability. The density of lateral roots was found to remain more or less constant over a range of nitrate supply; however, the elongation rate of lateral roots was suppressed by nitrate supply. Heterogeneous supply of nitrate was found to induce the density of lateral roots in the close regions. In many species, small regions with high concentration of nutrients have been reported to stimulate local proliferation of lateral roots (Farley and Fitter, 1999) [8]. Drew *et al.* (1973) [5] have shown that locally applied nitrate stimulated lateral root growth within the nutrient rich zone but not elsewhere. Our results support this reasoning, as we have observed reduction in the Basal Zone Length in comparatively higher N treatments, indicating promotion of growth of lateral roots under the stimulus of higher N availability. This is also attributed to be the direct or indirect result of the change in the metabolic activity of particular segment of root. N source in the form of nitrate ions promotes the elongation of lateral root (Zhang and Forde, 1998) [32]. Linkohr *et al.*

(2002) [16] reported that lateral root density in nitrate rich patch increased by approximately one lateral root per every cm of the primary root as compared to the homogenous control. They further observed that the lateral root density was very high in patches with more nitrate. They argued that lateral root growth on low nitrate was limited by starvation and in the high nitrate by inhibition. Along with this, it was also recorded that uniformly high nitrate supply suppressed the growth of primary root, which has been observed in some other studies also (Zhang and Forde, 1998; Williamson *et al.*, 2001) [32, 31].

References

1. Araya T, Miyamoto M, Wibowo J, Suzuki A, Kojima S, Tsuchiya YN *et al*. CLE-clavata1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. Proceedings of the National Academy of Sciences. 2014; 111:2029-2034.
2. Bidel L, Pagès L, Riviere L, Pelloux G, Lorendeau J. Mass Flow Dyn i: A carbon transport and partitioning model for root system architecture. Annals of Botany. 2000; 85:869-886.
3. Bhandari MM. Flora of the Indian desert. MPS Repros, Jodhpur, 1978.
4. Chevalier F, Pata M, Nacry P, Doumas P, Rossignol M. Effects of phosphate availability on the root system architecture: Large-scale analysis of the natural variation between *Arabidopsis* accessions. Plant, Cell & Environment. 2003; 26:1839-1850.
5. Drew M, Saker L, Ashley T. Nutrient supply and the growth of the seminal root system in barley: I. The effect of nitrate concentration on the growth of axes and laterals. Journal of Experimental Botany. 1973; 24:1189-1202.
6. Eissenstat DM. Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition. 1992; 15:763-782.
7. Evans JR, Terashima I. Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. Plant and Cell Physiology. 1988; 29:157-165.
8. Farley R, Fitter A. The responses of seven co-occurring woodland herbaceous perennials to localized nutrient-rich patches. Journal of Ecology. 1999; 87:849-859.
9. Fitter A. An architectural approach to the comparative ecology of plant root systems. New Phytologist. 1987; 106:61-77.
10. Garnett T, Conn V, Kaiser BN. Root based approaches to improving nitrogen use efficiency in plants. Plant, Cell & Environment. 2009; 32:1272-1283.
11. Giehl RF, Gruber BD, Wirén N. von. It's time to make changes: Modulation of root system architecture by nutrient signals. Journal of Experimental Botany. 2014; 65:769-778.
12. Jaramillo RE, Nord EA, Chimungu JG, Brown KM, Lynch JP. Root cortical burden influences drought tolerance in maize. Annals of Botany. 2013; 112:429-437.
13. Jordan MO, Harada J, Bruchou C, Yamazaki K. Maize nodal root ramification: Absence of dormant primordia, root classification using histological parameters and consequences on sap conduction. Plant and Soil. 1993; 153:125-143.
14. Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A *et al*. Analysis of the root system architecture of *Arabidopsis* provides a quantitative readout of crosstalk between nutritional signals. The Plant Cell. 2014; 26:1480-1496.
15. Lima JE, Kojima S, Takahashi H, Wirén N. von. Ammonium triggers lateral root branching in *Arabidopsis* in an ammonium transporter1; 3-dependent manner. The Plant Cell. 2010; 22:3621-3633.
16. Linkohr BI, Williamson LC, Fitter AH, Leyser HO. Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. The Plant Journal. 2002; 29:751-760.
17. Liu Y, Lai N, Gao K, Chen F, Yuan L, Mi G et al. Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in *Arabidopsis* thaliana. *PLoS One*, 2013; 8: e61031.
18. Lynch J, Marschner P, Rengel Z. Effect of internal and external factors on root growth and development. In: Marschner's Mineral Nutrition of Higher Plants, Elsevier, 2012, 331-346.
19. Lynch JP. Steep, cheap and deep: An ideotype to optimize water and n acquisition by maize root systems. Annals of Botany. 2013; 112:347-357.
20. McCully M. How do real roots work? (Some new views of root structure). Plant Physiology. 1995; 109:1.
21. McCully ME. Roots in soil: Unearthing the complexities of roots and their rhizospheres. Annual Review of Plant Biology. 1999; 50:695-718.
22. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum. 1962; 15:473-497.
23. Pan W, Jackson W, Moll R. Nitrate uptake and partitioning by corn (*Zea mays* l.) root systems and associated morphological differences among genotypes and stages of root development. Journal of Experimental Botany. 1985; 36:1341-1351.
24. Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E *et al*. The *Arabidopsis* nrt1. 1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proceedings of the National Academy of Sciences. 2006; 103:19206-19211.
25. Rich SM, Watt M. Soil conditions and cereal root system architecture: Review and considerations for linking Darwin and weaver. Journal of Experimental Botany. 2013; 64:1193-1208.
26. Robinson D. The responses of plants to non-uniform supplies of nutrients. New Phytologist. 1994; 127:635-674.
27. Robinson D, Hodge A, Griffiths BS, Fitter AH. Plant root proliferation in nitrogen–rich patches confers competitive advantage. Proceedings of the Royal Society of London. Series B: Biological Sciences. 1999; 266:431-435.
28. Sullivan WM, Jiang Z, Hull RJ. Root morphology and its relationship with nitrate uptake in kentucky bluegrass. Crop Science. 2000; 40:765-772.
29. Wang H, Inukai Y, Yamauchi A. Root development and nutrient uptake. Critical Reviews in Plant Sciences. 2006; 25:279-301.

30. White PJ, George TS, Dupuy LX, Karley AJ, Valentine TA, Wiesel L *et al*. Root traits for infertile soils. Frontiers in Plant Science. 2013; 4:193.
31. Williamson LC, Ribrioux SP, Fitter AH, Leyser HO. Phosphate availability regulates root system architecture in *Arabidopsis*. Plant Physiology. 2001; 126:875-882.
32. Zhang H, Forde BG. An *Arabidopsis* mads box gene that controls nutrient-induced changes in root architecture. Science. 1998; 279:407-409.
33. Zhang H, Jennings A, Barlow PW, Forde BG. Dual pathways for regulation of root branching by nitrate. Proceedings of the National Academy of Sciences. 1999; 96:6529-6534.