Exploring half root-stress approach: current knowledge and future prospects

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ABSTRACT
A half-root stress is a portion of the root system exposed to treatment while the remaining half portion kept under normal conditions. A half-root stress including half-root drought stress, half-root nutrient stress, and half-root salinity stress has become a general approach to improve plant performance and adaptability. Plants produce some chemical signals in stressed part of root, and other parts sense these signals to improve the acclimation and adaptive responses to environmental stresses. Plants adapt the compensatory functions and discriminate the systemic and local regulatory mechanisms, but the understanding of these mechanisms is controversial. Chemical signals (Abscisic acid, sap pH, cytokinins, content of malate, amino acid, and ureide) have been involved in root to shoot signaling under half-root stress. Furthermore, naturally appeared half-root stress in intercropping systems could be an additional attribute of half-root stress approach. Therefore, much more study is required to elaborate its acceptability in intercropping. In this review, we summarized the current knowledge and identified some key future researches areas regarding half-root stress approach.

Introduction
To encounter upcoming challenges in crop production triggered by changes in global climate, plant breeders and researchers join hands to develop high yield crops with better potential to withstand a variety of biotic and abiotic stresses (Koevoets, Venema, Elzenga & Testerink, 2016). However, the general focus of breeding efforts in improving crop yield is on above ground and shoot-related phenotypes, but roots hidden the half of plant are still a neglected portion of crop improvement. Roots are essential for plant adaptation and productivity, but less studied due to the difficulty of observing them during plant life cycle. Plant roots have several important functions such as uptakes of water and nutrients, formation of symbiotic relationship with microbes in the rhizosphere, anchoring the plant in the soil and storage organs (Khan, Gemenet & Villordon, 2016). Given roots playing the crucial role in the establishment and performance of plants, researchers have started ‘the second green revolution’ to explore the possibility of yield improvements through optimization of root systems (Iqbal et al, 2018a).

A half-root stress is a general approach that includes half-root drought stress (HRD), half-root nutrient stress (HRN), and half-root salinity stress (HRS). In this technique, roots are divided into two segments by placing them in adjacent containers or pots under different treatments (Figure 1). For example, HRD stress which includes regulated deficit irrigation (RDI) and partial root zone drying (PRD). RDI is a technique in which irrigation water is withheld to induce minor stress that has minimal effects on the yield. This technique commonly used in horticultural industry to improve product quality with efficient use of irrigation water (Sun, Feng & Liu, 2013). Whereas the PRD technique simply requires drying of one half of the root zone and leaving the other half wet. This technique significantly improves water use efficiency (WUE) of field and fruit crops, increases canopy vigor and maintains yields. PRD has an advantage over RDI in terms of increasing WUE and maintaining crop yield (Parviz, Sepashkah & Ahmadi, 2016). Comparison of PRD and RDI shows clear differences of leaf water relations, WUE, yield and fruit quality in crops receiving the same amounts of irrigation (Hapsari, Poerwanto, Sopandie & Santosa, 2018).

PRD is based on two theoretical assumptions: (1) a small narrow stomatal opening may reduce water loss, (2) root sourced signal from dry part of the root to shoot for partial stomata closure (Kang & Zhang, 2004). Photosynthetic rate and transpiration show saturation and linear response as stomata open in fully irrigated...
plants. However, plants growing under PRD would reduce water loss by narrowing stomatal opening but would have little effect on photosynthetic rate (Yan, Sun, Song & Liu, 2012). Consequently, this approach proves to increase plant WUE. The basic purpose of other approaches is same to avoid salinity stress and save fertilizers. This paper aims to review some results and experimental conclusions under half-root stress systems. This information will not only provide future guidance for half-root systems practitioners in arid and semiarid regions but also identify the knowledge gaps that will be beneficial to future research framework.

HRD stress approach and its wide application

It is a novel irrigation strategy that fulfills the actual need of plants and minimizes the water loss hence maximizes the crop productivity. Earlier work revealed this technique as water saving in many crops (Table 1). It simply requires drying of one-half of the root zone and leaving the other half-wet. Several scientists have used this approach for horticultural crops and fruit trees to improve WUE and eventually quality (Wang, Liu & Richardt Jensen, 2012; Yactayo et al., 2013; Zhou, Kang, Li & Zhang, 2008). To the best of our knowledge, numerous researches have done on grapevine species because of production frequently dependent on irrigation (Fuentes et al., 2014; Intrigliolo & Castel, 2009; Romero et al., 2015; Zhou et al., 2008). HRD stress as a water saving strategy may mitigate the impact of climate change on rising temperature and decreasing precipitation in the water scarcity areas. However, increase greenhouse gases emissions are considered the future predictions in a climate change scenario, and therefore, elevated CO2 concentrations and water scarcity will be an additional challenge for HRD stress approach. Very recently, study with elevated CO2 concentrations indicated the higher photosynthetic rate and grain yield as well as water productivity in maize plants under RDI than in full irrigation (Li, Kang, Zhang, Li & Lu, 2018). These results open a new direction to test the efficiency of HRD stress approach in specific agro-ecological conditions and under different environmental variables.

Feed-forward mechanism on HRD stress of plants

Previous studies have revealed that crop water requirement reduced by irrigating reduced root zone volume. Plants are able to detect the soil drying and reduce water use by regulating the physiological and biochemical responses in dry part of the root zone. Satisfactory water status is maintained in aerial parts of the plants and dry part of the root system can survive through fully hydrated parts of roots (Yan et al., 2012). Generally, roots in a given environment grow fast if other roots on the same plant have less access to resources (Langer, Syafuddin, Steinellner, Puschenreiter & Wenzel, 2010). Generalized responses of roots when they encounter a resource-rich patch of soil include increased resources uptake and life span, and changes in morphology (Xiaofei, Liang & Jianbin, 2016). The size of the aboveground portion of a plant is generally proportional to the volume of the root zone (Van Noordwijk, Lawson, Hairiah & Wilson, 2015). The expansion of aboveground
| No | Plants | Variety | Region | Experiment nature | Treatments | Water reduction | Reference |
|----|--------|---------|--------|-------------------|------------|----------------|-----------|
| 1  | Grapevine | Sultana | Australia | Pot experiment | Partial root-zone irrigation | – | (Stoll, Lovesy & Dry, 2000) |
|    |         |         |         |                   |            |                |           |
|    | Tempranillo | Valencia, Spain | Vineyard, field experiment | Irrigation amount and partial root-zone drying (PRD) | – | (Intrigliolo & Castel, 2009) |
|    |         | Northwest China | Vineyard, field experiment | Partial root-zone drip irrigation | – | (Zhou et al., 2008) |
|    | Shiraz and Tempranillo | Australia and Spain | Field experiment | Deficit irrigation and partial root-zone drying | – | (Fuentes et al., 2014) |
|    | Monastrell | Jumilla, Murci, Spain | Field experiment | Partial root-zone irrigation and regulated deficit irrigation | – | (Romero et al., 2015) |
| 2  | Potato | Liseta | Serbia | Pot experiment | Well-watered and half-stressed split-root | 33–42% | (Jovanovic et al., 2009) |
|    |         | Agria and Ramos | Peru | Field experiment | Partial root-zone drying irrigation, Full Irrigation and a deficit irrigation | – | (Ahmadi, Agharezaee, Kamgar-Haghighi & Sepaskhah, 2014) |
|    |         | Folva | Taastrup, Denmark | Glasshouse pot experiment | Full irrigation (FI), deficit irrigation (DI) and partial root-zone drying (PRD) | 37% | (Liu et al., 2006a) |
|    |         | Folva | Denmark | Field experiment | Full irrigation (FI), deficit irrigation (DI) and partial root-zone drying (PRD) | 30% | (Shahnazari et al., 2008) |
|    |         | Folva | Denmark | Pot experiment | Partial root-zone drying | 30% | (Liu et al., 2006b) |
|    | Tomato | Petopride | Palmerston North, New Zealand | Wooden boxes experiment | Partial root zone drying | 50% | (Zegbe, Behboudian & Clothier, 2004) |
|    |         | Sunpak | Germany | Compost filled seed trays experiment | Regulated deficit irrigation and partial root-zone drying | – | (Savić et al., 2008) |
|    |         | F1 Fantastic | Mexico | Greenhouse trays experiment | Partial root zone drying | 46% | (Campos, Trejo, Pena-Valdivia, Ramirez-Ayala & Sánchez-Garcia, 2009) |
|    |         | Cedrico | Denmark | Plastic greenhouses experiment | Full irrigation (FI), deficit irrigation (DI) and partial root-zone drying (PRD) | – | (Kirda et al., 2004) |
|    |         | Deja | Shaanxi, China | Pot experiment | Partial root-zone irrigation and deficit irrigation | – | (Wang et al., 2012) |
| 4  | Hot pepper | Toro F1 | Mexico | Greenhouse trays experiment | Partial root-zone drying | 40% | (Kang, Zhang, Hu & Li & Jere, 2001) |
|    |         | Cedrico | Denmark | Glasshouse experiment | Partial root-zone irrigation and deficit irrigation | 50% | (Dorji, Behboudian & Zegbe-Dominguez, 2005) |
|    |         | - | Shaanxi, China | Glasshouse experiment | Controlled alternate drip irrigation on partial roots | 16% | (Gençoğlan, Altunbey & Gençoğlan, 2006) |
|    | Common bean | Bronco | Morocco | Pot experiments | Partial root drying (PRD) and regulated deficit irrigation (RD) | – | (Wakrim, Wahbi, Tahi, Aganchich & Serraj, 2005) |
| 6  | Green Bean | Strike | Kahraman Maras, Turkey | Field experiment | Subsurface drip irrigation and partial root-zone drying irrigation | 16% | (Gençoğlan, Altunbey & Gençoğlan, 2006) |
|    |        | Bellamy | Australia | Field experiment | Partial root-zone drying | – | (Hutton & Lovesy, 2011) |
| 7  | Mango | Chok Anan | Thailand | Field experiment | Deficit irrigation and partial root-zone drying | – | (Spreer et al., 2007) |
| 8  | Maize | Shandan No. 9 | Shaanxi, China | Pot experiment | Split root system | 34.4–36.8% | (Kang, Liang, Hu & Zhang, 1998) |
| 9  | Cotton | Xinluzao No. 7 | Northwest, China | Greenhouse experiment | Partial root-zone irrigation | 30% | (Du, Kang, Zhang & Hu, 2006) |
| 10 | Soybean | Xin K4 | China | Field experiment | Partial root-zone irrigation (PRI) | 30% | (Tang, Li & Zhang, 2005) |
| 11 |        | Williams | Tehran, Iran | Field experiment | Partial root drying | 50% | (Sarai Tabrizi, Parsinejad & Babazadeh, 2012) |

: not measured
growth is generally attributed to increased nutrient uptake and hormone translocation from the root system. The ability of roots to absorb water and nutrients improved when the roots were partially watered (Manjunath, Laxman, Upreti & Raghupathi, 2018).

Such feed-forward mechanism produces some chemical signals in dry soil, which ultimately reduces stomatal conductance, transpiration rate, and plant shoot growth (Tardieu, 2016). Reduced transpiration under HRD stress lowers the xylem translocation as a result some chemicals may accumulate in root which attribute to decline degradation activity rather than to increase biosynthesis (Gil-Quintana et al., 2013). Ureide metabolite accumulation plays a regulatory role in nitrogen feedback inhibition of nitrogenase enzyme (Carter & Tegeder, 2016).

Plants produce some specific chemical signals in drying part of root to regulate shoot physiology with a concomitant increase in WUE and limited yield reduction (Yactayo et al., 2013). Such root sourced chemical signals are mainly an elevation of abscisic acid (ABA) concentration in dry part of root system (Puértolás, Conesa, Ballester & Dodd, 2014). ABA acts as signal transduction pathway that regulates many genes. These genes may express in response to specific environmental stresses (Lai et al., 2012). ABA induces the antioxidant genes expression encoding Cu, Zn, Mn, and Fe-superoxide dismutase (SOD) and catalase (CAT) (Trchounian, Petrosyan & Sahakyan, 2016). It shows that ABA is a source of oxidative stress in plants.

ABA increases the levels of reactive oxygen species (ROS) such as superoxide anion (\(O_2^-\)), hydroxyl radical (-\(\cdot\)OH), and hydrogen peroxide (\(H_2O_2\)). An absence of antioxidant defense system can severely damage the plant metabolism, for example, reactive oxygen species magnify the effects of water stress by affecting cell membrane properties of plants and cause oxidative damage to chlorophylls, lipids, proteins, and DNA, all of which contribute to cell death (Štolfa, Maronič, Pfeiffer & Lončarič, 2016). Plants mobilize antioxidant defense system to resist ROS. Major enzymes, which scavenge ROS, are superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), etc. (Figure 1). Half-root approach has found to activate the osmoprotectants and antioxidant system such as soluble peroxidases and superoxide dismutase (Liu, Song, Liu, Liu & Zhu, 2018). Similarly, Aganchich et al., (2007) noted a reduction in plant vegetative growth with the up regulation of enzymatic activities such as soluble peroxidases, superoxide dismutase, and insoluble peroxidase under partial root drying as compared to control treatment (Aganchich et al., 2007). It shows that half-root approach can alleviate the negative effects induced by water deficit on crops through regulation of physiological parameters.

Plant is a complex organism, hormones act as signals to regulate plant growth. Hormonal regulation and symbiotic relationships provide benefits for plants to overcome stress conditions (Beatriz, Manuel, María, María & Ricardo, 2016). Lu et al. (2015) treated half portion of root system with methyl jasmonic acid and the other one-half of the roots with rice water weevil. They observed that jasmonates are induced signals that increase root resistance against root herbivores and can reduce root damage by belowground feeders (Lu et al., 2015). Moreover, external application of jasmonic acid caused transduction of a signal from the treated roots to the shoot, leading to an increase in carbon allocation from the leaves to the untreated root tissue. Therefore, the response of some plant species may be the diversion of resources to safer locations (Henkes, Thorpe, Minchin, Schurr & Roese, 2008).

Overall effects of this approach may also depend on some additional changes in other chemical signals. For example, cytokinins (CKs) which plays antagonist role to ABA (Figure 1), consequently modify plant responses to drought stress (Wang et al., 2011). A research conducted by Beis & Patakas (2015) concluded a more obvious role of CKs in mediating stomatal responses in partial root zone drying plants. In addition, grain filling rate and grain weight of newly bred super rice inferior spikelets were found to be correlated with CKs levels (Zhang, Chen, Wang, Yang & Zhang, 2010). It might be possible that the ABA/CKs ratios in xylem sap are important for stress signaling. These biochemical responses can be utilized without affecting the crop growth physiology under water deficit conditions. However, the specific effects of this approach may depend on crop and crop root distribution, proportion of roots exposed to drying parts and soil water contents in the wet root zone. Still many considerations are needed before this system is used as an effective management irrigation technique for plants.

HRD stress to understand the regulation of nodulation and nitrogen fixation

Nodule formation is associated with symbiotic nitrogen fixation (SNF) bacteria. Capable plants (legumes) convert nitrogen from atmosphere into ammonia, which is then assimilated into amino acid, nucleotides, vitamin, flavones, and hormones. This process requires a lot of energy; therefore, nodulation is a strictly controlled process. The number of nodules is controlled by auto-regulation of nodulation (AON), which is complex root to shoot and shoot to root signaling loop. Earlier
reports based on half-root system have shown the systemic regulation of AON (Kassaw, Bridges & Frugoli, 2015). Pre-inoculation of one side of the half-root system has been exposed to inhibit subsequent nodule formation in the untreated side of the roots in several legumes species (Jeudy et al., 2010). Locally produced signal in the treated roots and/or nodules translocate to the shoots and same signal transmits again to the untreated roots to prevent excessive nodule formation (Figure 2). Molecular approaches have identified the key players in the systemic regulation of AON such as legume orthologs of Arabidopsis thaliana CLAVATA-like Leu-rich repeat receptor-like kinases (LRR-RLKs) (Imin, Patel, Corcilius, Payne & Djordjevic, 2018). Based on the evidence that interaction of CLE-family peptide CLV3 activates the A. thaliana CLAVATA, half-root system based experiments have shown two GmCLE genes (GmRIC1 and GmRIC2) involved in AON in soybean (Lim, Lee & Hwang, 2011).

Environmental factors such as drought have shown more effects on SNF in legumes. Numerous hypotheses have proposed to describe the reason behind the reduction of SNF rates under drought. There are several factors involved in the regulation of SNF under drought such as oxygen control, carbon limitation, and regulation of N-feedback (Arrese-Igor et al., 2011). Traditionally, it was thought that photosynthesis process is responsible for SNF regulation in legumes, which was proved wrong by Durand et al. in 1987 (Durand, Sheehy & Minchin, 1987). They highlighted the high sensitivity of this process by noticing the inhibition of SNF under moderate drought that occurred prior to any measurable drop in photosynthesis rates. Recent researchers used half-root system to demonstrate the local regulatory mechanisms controlling SNF in legumes under drought (Gil-Quintana, Larraínzar, Arrese-Igor & González, 2012). For instance, the content of malate (main C substrate used by bacteroids) only declined in the water-deprived half-root side associated with a reduction in sucrose synthase and apparent nitrogenase activity (Marino et al., 2007). Significant downregulation of SS gene expression and activity in water-deprived half-root side played a key role. These results provide evidence that SNF under drought is mainly regulated at the local level. Furthermore, Gil-Quintana et al. (2013) also observed the local regulation of SNF, and metabolic profiling of amino acid and ureide support the hypothesis.

Although half-root approach is a useful tool to study the systemic nodulation control and local inhibition of SNF, it has also identified factors that locally affect the number of nodule such as ABA (Biswas, Chan & Gresshoff, 2009) and iron concentration (Tang, Robson & Dilworth, 1990). Although much attention has been paid to half-root research but the nature of the signals involved and how they are perceived requires further research efforts. Half-root based studies will help to elucidate these unsolved questions in the future.

HRNs and salinity stress approach and plant responses

The effective use of nutrients has become an important component in the production of crops and fruit-bearing trees. Earlier work to investigate the vascularization response of three tomato cultivars to soil type, nutrient stress, and water stress has revealed that plants with nutrients stress showed maximum veins (Sanders, Cure, Dayton & Gardiner, 1996). Thus, application of all nutrients at the same time is a difficult task to maximize the growth and development of plant through optimum nutrient concentration and uptake (Abbasov, 2013).

The nutrients concentration above than optimum level is harmful to plants; therefore, split-root system may consider as a problem solving technique. It may feasible to know the plant status with respect to plant essential and beneficial nutrient elements. A split-root nutrient system is a similar phenomenon as localized fertilization in row crops. It involves the separation of plant roots into two parts with different concentration of nutrient media (Dener, Kacelnik & Shemesh, 2016). High- and low-nutrient concentration on both sides enables the plant to uptake optimum nutrients by absorbing desired quantities of nutrients.

The application of low quality water has increased due to high water demand for enhanced yield and profits. Therefore, it is practically important for arid region crops to understand the spatial pattern of nutrient uptake from soil that are irrigated with low quality of water (Melgar et al., 2009). Generally, soil salinity is spatially heterogeneous in saline lands and its range experienced by the plant roots can be large (Bazihizina, Barrett-Lennard & Colmer, 2012). It develops a gradient in and around the root zone; therefore, some roots are provided with nutrient rich and fresh water constantly, while others are exposed to salinity stress or to dehydrated soil layers (Corwin, Rhoades & Šimůnek, 2007).

Nutrient stress and ion toxicity lead to plant growth inhibition. Root water absorption is difficult under high salt concentration in soil and excessive salt accumulation in plant tissue causes ion toxicity (Munn & Tester, 2008). Plants decrease water uptake under salinity stress as the concentration of sodium chloride increases in the medium that affect the nutrient mobilization as well. However, split-root approach may be able to play a compensatory function. For instance, non-uniform soil
salinity increases water use of plant than uniform soil salinity (Bazihizina, Colmer & Barrett-Lennard, 2009). Plant roots restrict the water uptake from high-salinity area/zone; however, roots increase the water uptake from low-saline area/zone consequently, uptake of water remains unchanged by entire root and even the same mechanism with NO3 uptake with slight effect (Bazihizina et al., 2009; Flores, Angeles Botella, Martinez & Cerdá, 2002). The mangrove (Avicennia marina L.) seedlings grown in split-root experiment showed preferential freshwater uptake that also increased stomatal conductance (Reef, Markham, Santini & Lovelock, 2015).

Similarly, deficiency of Fe in a part of the root system caused a substantial increase in iron reductase activity and proton extrusion in the Fe supplied part (Wu et al., 2011). Furthermore, the phosphorus uptake in split-root system with treatment of –P +P was optimum as compared to –P –P and +P +P (Shen, Li, Neumann & Zhang, 2005). The concentration and uptake of phosphorus in low P-medium increased with increasing P-amounts to the sand section (Shu, Shen, Rengel, Tang & Zhang, 2005).

The question arises whether this kind of mechanism can be used to increase nutrient use efficiency (NUE) or not? Mainly, optimization of plant nutrition is an important aspect of plant science. Different morphological and physiological strategies have been developed by higher plants to boost nutrient acquisition and utilization, e.g. phosphorus (P) in P-limiting environments (Vance, Uhde-Stone & Allan, 2003). Cluster roots formation is an important phenomenon in plant development that improves the plant capacity to acquire soluble P from soil (Shen et al., 2005). In a split-root system of N rich and deficient treatment, local and systemic signals in roots and shoots are integrated, thereby stimulating nitrate acquisition in the nitrate-rich patch. The secreted small signaling peptide C-TERMINALLY ENCODED PEPTIDE 1 (CEP1) are produced by roots under N deficiency and transported to the shoot via the xylem, which leads to induction of the non-secreted small signaling peptides CEP DOWNSTREAM 1 (CEPD1) and CEPD2. CEP family peptides mediate up-regulation of nitrate transporter genes (NRT2.1, NRT3.1, and NRT1.1) in the distant part of the roots exposed to N-rich conditions to compensate for N deficiency (Figure 2). In the end, nitrate acquisition at the nitrogen-rich site of a split-root system was enhanced (Oh, Seo & Kim, 2018; Tabata et al., 2014). Hence, split-root system would have a better opportunity for plant to fulfill their nutrient needs. In addition, the use of reformulated fertilizers to make sure that nutrients are available as needed. If we are able to achieve this then NUE will be improved at little expense of fertilizer inputs (calculated as the yield per unit input).

Does the split-root system have possible effect on root growth induced by nutrient application, and what are the relevant functions of these roots? An application of localized, polypropylene, nonwoven fabric fertilizer produced maximum number of roots from root collar, subsequent in prompt shoot growth (Tworkoski, Daw & Glenn, 2003). The dry weight of split roots under nutrient stress was greater than that of control side, which indicates the different pattern of water absorption and assimilates accumulation of split stressed roots than that of control. Moreover, local deficiency of P suppressed S uptake and magnesium contents increased by the roots grown in the part with no Mg supply (Shen et al., 2005). Eventually, split-root system increases productivity due to increased water potential in one side of root system and increased enzyme activities (de Cássia Alves et al., 2018).

Based on the existing knowledge, split-root nutrients system is an approach to save fertilizers. Nevertheless, still finding the suitable nutrient concentration and location in the medium may affect the morphophysiological characteristics of plant. In addition, split-root salinity approach is not suitable to study the

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Figure 2. Schematic diagram of mechanisms to understand the local and systemic regulation of plant under half-root stress.
salinity effect on symbiosis because nodules and roots may not expose to uniform stress due to zones of variable osmotic potential created by migration of salts in rooting medium.

**Naturally appeared half-root stress in intercropping**

Intercropping has ecological, biological and socio-economic advantages. Maize and soybean relay strip intercropping system is widely practiced in southwest of China that provides higher yield (LER) and ecological values (water saving and drought-resistance) (Iqbal et al, 2018b). This system has minimum soil water loses and high water productivity (Rahman et al., 2017). However, the mechanism is still not entirely clear. Half-root stress appears naturally in intercropping system that gives us some enlightenment to elucidate the various advantages. Previous studies have shown that the field microenvironment exhibits changes for soybean plant, especially for the light and water conditions in maize and soybean intercropping systems. Better crop water use rate and uneven water distribution was found in maize-soybean strip intercropping system over sole cropping (Rahman et al., 2017). Soil moisture contents vary in narrow maize rows, maize and soybean rows, and soybean rows (Feng et al., 2015). Moreover, soil water content decreased gradually from soybean rows to maize rows in a single strip (Figure 3). Other similar studies found that soil volumetric water content and soil evaporation in maize-soybean relay intercropping systems showed decreasing trends in the order: maize row < maize-to-soybean row < soybean row (Lin et al., 2015; Rahman et al., 2017).

This phenomenon produces the hypothesis about water imbalance conditions (similar as half-root approach) in the field under maize-soybean strip intercropping system, and soybean plant (between point B and C) may suffer from different moisture contents on both sides (Figure 3). Water imbalance in maize-soybean strip intercropping system could activate the various water deficit stress mechanisms in soybean plant, which may induce morphological, physiological, and metabolomics changes in plants. According to our research, imbalanced water conditions with moderate water reduction can improve soybean seed-quality and drought-resistant genotypes can increase the soybean yield under intercropping systems (Iqbal et al., 2018a). Similarly, appropriate intercropping shading can improve the flavor quality of soybean seeds under maize-soybean intercropping system (Liu et al., 2016). Therefore, combined stresses (imbalance water deficit and shade) appear in intercropping systems could make the problem more complex and interesting. Split-root approach can provide better understanding of plant when studying the enzymatic, molecular, and metabolomics reactions under imbalance water deficit and shade conditions.

In addition, dynamic changes in soil water content during water imbalanced conditions may have impact on availability and movement of nutrients in soil profile. Reduced nitrogen application is useful for maize-soybean

![Figure 3](https://example.com/f3.png)

**Figure 3.** (I) Schematic representation of maize soybean intercropping, water, and fertilizer imbalance site. (II) Soil water contents of different spatial position in maize-soybean relay intercropping system. A stands for narrow maize rows, B stands for between maize and soybean, C represents the soybean lines while D shows the soybean sole crop. (III) Split-root approach as a tool to study the local or systemic nature of soybean processes of signal.
strip intercropping system. It increases nitrogen use efficiency and yield because mitigates the soil acidification in maize-soybean strip intercropping system (Yong et al., 2014). Soybean is usually not fertilized in this system, so that the fertilizer is mainly applied to the maize line (point B) (Chen et al., 2017). Interspecific competition for resource utilization is notable to affect crops performance in intercropping systems (Yang, Li, Wang, Wu & Zhang, 2013). Therefore, it indicates the similar fertilizer imbalance conditions in maize-soybean strip intercropping system. This phenomenon produces the kind of hypothesis about HRN. Half-root based studies will help to shed further light on these hypotheses in the future.

Intercropping could be an additional attribute of PRD. PRD maintains WUE and improves yield. The principal of this approach is alternating water in place and time to produce wet-dry cycles in different segments of the root system. However, how much root system needs to dry out to get this effect requires further research efforts. Moreover, do the different levels of moisture on both sides of root (moisture imbalance) have any effect on the yield and quality of soybean? In this view, a study could be initiated to examine the variation in morpho-physiology of soybean growing under split-root system with imbalance nutrient and moisture levels. Further, and more importantly, split-root approach could be useful when studying the local and systemic enzymatic, molecular, and metabolomics reactions providing better understanding of plant.

Conclusion

Combined half-root stress appears in nature and agricultural practice, which makes the problem more complex and interesting. It is a useful approach to study the feed-forward mechanism of plant. Plants produce some specific chemical signals in stressed part of root to regulate shoot physiology, e.g. activation of antioxidant defense system to resist HRD stress and compensation of half-deprived nutrient and salinity stress. Furthermore, this approach let plants to discriminate the systemic and local regulatory mechanisms that still need deep investigation, for instance, the systemic nodulation control and local inhibition of SNF. Continuing studies about physiology, metabolism and molecular levels will help us to understand the mechanisms deep, operating in half-root stress (water, nutrients, and salinity) grown plants. Understanding of these regulation processes could lead physiologist to analyze the local and systemic responses of plant more easily.

In addition, naturally appeared half-root stress in intercropping systems could be an additional attribute of partial root drying irrigation. However, it is crucial to know whether imbalance water, nutrients, and salinity in the root zone are acceptable or not? How much root system needs to dry out to get this effect requires further research efforts. Moreover, do the different levels of moisture and nutrients on both sides of root (moisture imbalance) have any effect on the yield and quality of cereal? Therefore, comprehensive study is required to understand the combined effects of imbalance water and nutrient deficits, and shade conditions under intercropping systems.

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