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Research article

Root exudation potential in contrasting soybean genotypes in response to low soil phosphorus availability is determined by photo-biochemical processes

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ABSTRACT

Low phosphorus (P) availability elicits efflux of organic substances viz. carboxylic acids, phenolics, proteins, amino acids, sugars and other low molecular weight compounds in many leguminous crops including soybean (*Glycine max* (L.) Merr.). The potential for root exudation varies widely among soybean genotypes, as synthesis and secretion of root exudates place additional burden on the carbon demand of the plant. Hence, efficient photosynthetic machinery may attribute to the differential root exudation potential of soybean genotypes in response to low soil P availability. An attempt was made to understand the varietal differences in photo-biochemical processes of soybean genotypes identified previously with contrasting root exudation potential under low P (Vengavasi and Pandey, 2016). Genotypes EC-232019 (P-efficient) and EC-113396 (P-inefficient) were grown in soil with low (2 mg P kg^{-1} soil) and sufficient (25 mg P kg^{-1} soil) P levels under natural environment and observations were recorded at anthesis. The genotype EC-232019 exhibited higher maximal carboxylation rate (V_{cmax}), maximal photosynthesis (A_{max}), apparent quantum efficiency (Φ), mesophyll conductance (g_m), triose phosphate utilization rate (TPU), photochemical quenching (qP) and electron transport rate (ETR), along with higher chlorophyll *a*, total chlorophyll and total carotenoid concentration as compared to the P-inefficient EC-113396. Low P-induced reduction in maximal electron transport rate (J_{max}) and Φ was higher in EC-113396 rather than EC-232019, suggesting superior photo-biochemical efficiency in the latter. The observed variation in P uptake and growth responses might be attributed in part to the improved photo-biochemical processes exhibited by the P-efficient genotype EC-232019.

1. Introduction

Soybean (*Glycine max* (L.) Merr.), a pulse-cum-oilseed crop, is cultivated in approximately 6% of the worlds' agricultural land (Goldsmith, 2008). India is the fifth largest producer of soybean in the world after the United States, Brazil, Argentina and China with an average productivity of 737 kg ha^{-1} (FAOSTAT, 2017). Soybean productivity is severely affected by various biotic and abiotic stresses, which is on the rise due to the vagaries of climate change.

Phosphorus (P) is a major yield-determining nutrient in legume-based cropping systems. The major problem with P nutrition is not the P content present in soil but its bioavailability to plants, as inorganic P gets immobilised in acid soils with Fe^{3+} and Al^{3+}, whereas in calcareous soils, P is fixed with Ca^{2+} (Liao et al., 2006). Plants adapt to low P soils through several mechanisms including exudation of carboxylic acids such as malate, citrate and oxalate (Dong et al., 2004; Liao et al., 2006; Jakkeral and Kajjidoni, 2011; Liang et al., 2013), secretion of phosphatase and nuclease enzymes (Nuruzzaman et al., 2005; Wang et al., 2009; Gaxiola et al., 2011). Low molecular weight exudates like carboxylic acids, sugars, phenolics and amino acidshave a major role in enhancing P acquisition (Carvalhais et al., 2011; Vengavasi et al., 2016). About 30% of fixed carbon is released in the form of exudates during P deficiency (Khorassani et al., 2011). Higher root exudation potential demands that plants exhibit superior source strength under

Abbreviations: Φ, apparent quantum efficiency; NPQ, alternative non-photochemical quenching; C_c, chloroplastic CO_2 concentration; R_d, dark respiration; ETR, electron transport rate; C_i, intercellular CO_2 concentration; LCP, light compensation point; F_m and F_m', maximal fluorescence; F_o and F_o', minimal fluorescence; A_{max}, maximal photosynthesis; g_m, mesophyll conductance; V_{cmax}, maximal carboxylation rate; J_{max}, maximal electron transport rate; g_m, mesophyll conductance; qN, non-photochemical quenching; qP, photochemical quenching; P_N, photosynthesis; PPFD, photosynthetic photon flux density; PSII, photosystem II; P, phosphorus; A, rate of photosynthesis; g_s, stomatal conductance to water vapour; F_s, steady state fluorescence; TPU, triose phosphate utilization rate

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stress in terms of better leaf area, photosynthate production and assimilate partitioning. Crop species/genotypes with higher exudation of P mobilising compounds such as carboxylates and phosphatases divert more carbon towards their biosynthesis, in addition to that required for normal growth and development of the plant. Hence, the photo-biochemical processes influencing photosynthetic capacity determine the plants' adaptive responses to low soil P availability.

Photosynthesis (P_N) is influenced by both stomatal and non-stomatal (mesophyll) factors. Rate of P_N is positively correlated to chlorophyll *a* fluorescence-derived traits such as maximum quantum efficiency of PSII photochemistry (F_v/F_m) (Yin et al., 2010). The equilibrium between light capture and metabolite production in plants is tightly regulated through a network of signal transduction pathways, hence slight fluctuations in the environmental conditions can drastically affect the plant metabolome. Limitations on the stomatal conductance to water vapour (g_s), amount of Rubisco enzyme and/or its activation, regeneration of Calvin cycle intermediates and photochemical quenching (qP) mechanisms are also known to influence the photosynthetic capacity of a plant under stress. P deficiency diminishes the plants' capacity for CO_2 assimilation leading to excessive absorption of incident light energy (Laisk and Loreto, 1996). A large fraction of this excess energy is dissipated through processes other than CO_2 fixation, collectively referred to as non-photochemical quenching (qN). Up regulation of qN mechanisms, particularly the photorespiratory cycle caused drastic reduction to Fv/Fm and qP in P-deficient soybean leaves (Singh and Reddy, 2015).

Light saturation point decreased by 37% due to P deficiency, while apparent quantum efficiency (Φ), light-saturated maximal photosynthesis (A_{max}) and maximal carboxylation rate (V_{cmax}) reduced by 29, 51 and 83%, respectively in soybean (Lauer et al., 1989). P deficiency in common bean (*Phaseolus vulgaris*) reduced P_N and g_s, while intercellular-to-ambient CO_2 ratio (C_i/C_a) did not vary much, implying excess source activity rather than an increased sink demand (Lima et al., 1999). Such responses of P_N and associated traits to P nutrition has been reported in several crops including soybean (Fredeen et al., 1989), wheat (*Triticum aestivum*), maize (*Zea mays*) and sunflower (*Helianthus annus*) (Jacob and Lawlor, 1991), common bean (Nielsen et al., 2001), rice (*Oryza sativa*) (Xu et al., 2007), potato (*Solanum tuberosum*) (Fleisher et al., 2012) and cotton (*Gossypium hirsutum*) (Singh et al., 2013). Nevertheless, whether the photo-biochemical efficiency varies among crop genotypes with contrasting carbon (sink) demand under low P availability remains to be tested. Non-destructive techniques to measure chlorophyll fluorescence offer exciting opportunities to probe into the photosynthetic apparatus of chloroplasts (Baker et al., 2007). Simultaneous measurement of gas exchange and chlorophyll *a* fluorescence helps resolve the factors that determine the balance between photosynthetic electron transport, CO_2 assimilation and photorespiration. This would aid in better understanding of the plants' P efficiency and metabolic alterations determining photosynthetic productivity.

Exploring the physiological mechanisms enabling sustained growth and development in P-efficient soybean genotypes may advance our knowledge regarding trait selection in other legume species. It was hypothesised that genotypes exhibiting higher root exudation potential under low P stress possess superior photo-biochemical efficiency to meet their higher carbon demand. From our previous experiments, soybean genotypes, EC-232019 (P-efficient) and EC-113396 (P-inefficient), contrasting in their root exudation capacity under low soil P was identified (Vengavasi and Pandey, 2016). These genotypes differed at physiological and molecular levels in response to low P stress. The root traits, total P uptake, exudation of carboxylates and activity of enzymes and/or genes involved in their synthesis and efflux as well as whole root proteome differed significantly (Vengavasi et al., 2017). In the present study, we investigated the influence of low soil P availability on gas exchange parameters and chlorophyll *a* fluorescence traits in contrasting soybean genotypes in order to relate the photo-biochemical efficiency with root exudation potential.

2. Materials and methods

2.1. Plant material and growth conditions

Soybean (*Glycine max* (L.) Merr.) genotypes EC-232019 (P-efficient) and EC-113396 (P-inefficient) contrasting in P uptake efficiency were grown in natural environment to assess the growth, photosynthetic efficiency and yield traits under low and sufficient P availability. Recommended package of practices were followed for growing soybean plants. The seeds were soaked overnight in deionized water and treated with *Bradyrhizobium japonicum* culture and sown in earthen pots (30 cm diameter) containing 12 kg sandy loam soil. The pH (soil:water::1:5) and electrical conductivity of the soil was 8.2 and 0.174 mS m^{-1} respectively. Available P extracted by Olsen method (Olsen et al., 1954) in the soil was 2 mg P kg^{-1} soil. Additionally, single super phosphate was applied at the recommended rate of 25 mg P kg^{-1} soil to create sufficient P level. Recommended dose of nitrogen and potassium for soybean were added to the soil through urea and muriate of potash, respectively. Four seeds were sown in each pot and upon emergence of the first trifoliate leaf, thinned to retain two healthy and uniform plants per pot. Four replications with six pots each were maintained for all treatments. To minimize heterogeneity, pots were repositioned during the experimental period. All observations were recorded at anthesis (reproductive stage R6), which is the metabolically active stage with higher demand for energy and P nutrition.

2.2. Growth and tissue P status

Total leaf area (cm^2 $plant^{-1}$) was measured using leaf area meter (Li-Cor 3100, Li-Cor, Lincoln, NE, USA). Area and dry weight of uppermost fully expanded leaves was recorded to determine the specific leaf area (SLA) expressed as cm^2 g^{-1}. Plants were uprooted and separated into leaf, stem and root to determine biomass accumulation (g $plant^{-1}$) and root-to-shoot ratio. Leaf, stem and root P concentration (%) was determined (Murphy and Riley, 1962) after digestion of dry tissue with di-acid mixture (HNO_3:$HClO_3$::9:4). Total P content calculated from the tissue P concentration and dry weight of leaf, stem and root was expressed as mg P $plant^{-1}$.

2.3. Gas exchange and chlorophyll a fluorescence measurements

Measurements were made on the central one-third region of uppermost fully expanded leaves between 0800 and 1130 h, using Li-Cor 6400*xt* Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA) with an integrated fluorescence chamber (Li-Cor 6400-40 mounted with Leaf Chamber Fluorometer). Measurements were recorded within a leaf area of 2 cm^2 at photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1} and block temperature of 28 °C. Reference CO_2 concentration of 380 μmol mol^{-1} was maintained at a constant flow rate of 500 ml min^{-1} using 12 g Li-Cor CO_2 cylinders. Relative humidity varied between 45 and 55%.

Photosynthetic CO_2 response curve was obtained by changing the CO_2 concentration entering the leaf cuvette as 400, 300, 200, 100, 50, 400, 400, 600, 800 and 1000 μmol mol^{-1}, with other variables at a constant rate and recording the steady state P_N at each CO_2 level (stability factor > 0.75). The input parameters (P_N and intercellular CO_2 concentration) were used to fit the model using a non-linear curve fitting utility (Sharkey et al., 2007). Chloroplastic CO_2 concentration (C_c) was calculated from the equation:

$$C_c = C_i - \left(\frac{A}{g_m}\right)$$

where A: rate of photosynthesis, C_i: intercellular CO_2 concentration, g_m: mesophyll conductance. The Farquhar-type C_3 photosynthetic model (Farquhar et al., 1980) was used to derive the maximal carboxylation