

## Article

# Physiological basis of combined stress tolerance to low phosphorus and drought in mungbean core set derived from diverse germplasm

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**Abstract:** To understand the physiological basis of tolerance to combined stresses to low phosphorus (P) and drought in mungbean (*Vigna radiata* (L.) R. Wilczek), a core set of 100 accessions were evaluated in hydroponics at sufficient (250  $\mu$ M) and low (3  $\mu$ M) P, and exposed to drought (dehydration) stress. The principal component analysis and ranking of accessions based on relative values revealed that IC280489, EC397142, IC76415, IC333090, IC507340 and IC121316 performed superior while IC119005, IC73401, IC488526 and IC325853 performed poorly in all treatments. Selected accessions were evaluated in soil under control (sufficient P, irrigated), low P (without P, irrigated), drought (sufficient P, withholding irrigation) and combined stress (low P, withholding irrigation). Under combined stress, a significant reduction in gas exchange traits (photosynthesis, stomatal conductance, transpiration, instantaneous water use efficiency), P uptake in seed and shoot was observed under combined stress as compared to individual stresses. Among accessions, IC488526 was most sensitive while IC333090 and IC507340 exhibited tolerance to individual or combined stress. The water balance and low P adaptation traits like membrane stability index, relative water content, specific leaf weight, organic acid exudation, biomass, grain yield and P uptake can be used as physiological markers to evaluate for agronomic performance. Accessions with considerable resilience to low P and drought stress can be either used as 'donors' in *Vigna* breeding program or cultivated in areas with limited P and water availability or both.

**Keywords:** Mungbean; low phosphorus; drought stress; organic acid exudation; photosynthetic rate; relative water content; membrane stability index; stress susceptibility index; principal component analysis ranking

## 1. Introduction

Mungbean (*Vigna radiata* (L.) R. Wilczek) is a short duration pulse crop grown by marginal and poor farmers as a grain and fodder crop, primarily in the arid and semi-arid regions of India that are prone to numerous abiotic stresses and greatly hampers seed yield [1]. Mungbean is preferred for its high nutritional value and for improving soil fertility. Phosphorus (P) is a non-renewable resource and the second most important macronutrient for plant growth and development after nitrogen (N). Deficiency of P has deleterious effects on plant growth, development and reproduction. Limitation in

plant growth and seed yield under P deficiency is mainly attributed to its heterogenous distribution and low mobility in soil. Most of the world's arable lands exhibits high P retention potential that leads to poor bioavailability of P for plant uptake [2]. It is generally accepted that the uptake of P by crop plants is reduced in limited moisture condition through changes in mass flow and diffusion, reduced root proliferation, and by altered P uptake capacity [3-6]. Drought can depress plant growth by reducing N and P uptake, transport and their redistribution [7]. Under water deficient condition, P application can enhance root growth, nutrient uptake and water use efficiency (WUE) resulting in increased yield [8]. Drought can be ameliorated by increasing the availability of N, P, and potassium (K) that leads to higher root proliferation, water uptake, and WUE [9,10].

P plays essential roles in energy metabolism, biosynthesis of nucleic acids, membranes phospholipids, cellular signal transduction and regulation of several key enzymes [11]. P plays a role in improving root growth, maintaining cell turgidity with high water potential, and increases the stomatal conductance with higher WUE during moisture deficit condition [12]. Optimum P nutrition can contribute to a controlled and proper adjustment of the physiological, morphological, and biochemical processes of a plant to promote growth. Enhanced growth rate at higher P levels could be attributed to the increase in photosynthetic rate associated with the increase in stomatal conductance [13] as reported in cotton (*Gossypium hirsutum*) and Rhodes grass (*Chloris gayana*) under water deficit stress [14,15]. Tolerance to low soil P availability is associated with increased P acquisition efficiency which is achieved by modification in root architecture capable of foraging the nutrients from the upper soil layer [16-18]. P deficiency in rice caused a rapid decline in chlorophyll *a* content, while chlorophyll *b* decreased gradually over the period of P deprivation [19]. The reduction in chlorophyll content with moisture stress level have also been reported in common bean (*Phaseolus vulgaris*) [20], cluster bean (*Cyamopsis tetragonoloba*) [21], and mungbean [22].

The relative water content (RWC) is one of the key traits associated with drought stress and shows reduction in response to drought as reported in several crops such as soybean (*Glycine max*) [23,24], alfalfa (*Medicago sativa*) [25], and mungbean [22,26,27]. Another trait, excised leaf weight loss (ELWL), is often used as a selection criterion for drought tolerance in many crops. Genotypic variation for ELWL can be due to both the residual water loss from stomatal complex and epicuticular transpiration as reported among blackgram genotypes [28]. Mungbean genotypes with contrasting ELWL were explored for the variation in physiological and molecular responses to water deficit which revealed that efficient stomatal regulation was found in genotypes with low ELWL [29]. Further, a negative correlation between moisture retention capacity (MRC) and stomatal conductance was reported in common bean [30]. Under drought, a significantly positive correlation between MRC and yield attributes was reported in wheat [31]; on the contrary, a poor correlation between MRC and seed yield or dry matter was observed in *Phaseolus vulgaris* [32]. The negative effect of moisture deficit on yield and its attributes are well reported in legume crops [27,33,34]. Since P nutrition plays an important role in pod filling and thereby enhancing seed yield [35], so it is recommended to apply P under drought stress as observed in as cluster bean [36,37], cowpea (*Vigna unguiculata*) [38], chickpea (*Cicer arietinum*) [39], and soybean [40,41].

Generally, mungbean is cultivated under rainfed condition with minimal or without application of fertilizers. Moisture deficit affects soil P mobility towards the root while P deficiency influences growth and physiology of plants. Therefore, identification of mungbean accessions possessing physiological mechanisms to acquire and utilize P from soil having less soil moisture in rainfed regions would be useful in improving mungbean seed yield. In the present study, efforts were made to phenotype the core set selected from 1232 diverse mungbean accessions, in hydroponics under controlled condition followed by thorough physiological evaluation of selected accessions to low P and drought stress in soil. The aim was to identify the traits that could be used as physiological markers, and the 'donor' accessions may be included in the *Vigna* breeding program to develop varieties with enhanced water and P use efficiencies for cultivation under limited availability of water or P or both.

## 2. Materials and Methods

The core set comprising 100 accessions (Suppl. Table 1) was selected from a total of 1232 diverse mungbean germplasm [42]. These diverse germplasm were scored for the morpho-physiological and yield traits in the field experiment located at Issapur Farm, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India between latitudes 28°32'11" N and 28°42'45" N; longitudes 76°49'41" E and 77°02'53" E; 218 m above mean sea level (Suppl. Fig. 1a,b,c). Two separate experiments were conducted on the core set to investigate the effect of low P and drought on growth and physiology of mungbean.

### 2.1 Experiment 1: Screening in hydroponics

The core set was phenotyped in hydroponics at Controlled Environment Facility, Division of Plant Physiology, ICAR-IARI, New Delhi. Seeds of mungbean were surface sterilized with 0.1% HgCl<sub>2</sub> and rolled in moistened germination towel. Upon appearance of cotyledonary pair of leaves after five days, six seedlings were transferred to nutrient medium in each treatment. Seedlings were grown with two P levels: sufficient (250 µM) and low P (3 µM). The composition of the nutrient solution was: MgSO<sub>4</sub>·7H<sub>2</sub>O (1 mM), CaCl<sub>2</sub>·2H<sub>2</sub>O (1.65 mM), K<sub>2</sub>SO<sub>4</sub> (0.7 mM), Fe-Na-EDTA (0.03 mM), Urea (2.5 mM) with micronutrients H<sub>3</sub>BO<sub>3</sub> (2.9 µM), MnSO<sub>4</sub>·H<sub>2</sub>O (2.8 µM), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.5 µM), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.47 µM) and Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O (0.6 µM). The pH of the nutrient solution was adjusted between 6.0 and 6.2 (using either 1.0 N KOH or 1.0 N HCl). To find out the sufficient and low P levels, a preliminary experiment was conducted using two accessions (IPM 2-3 and MH 421) with different P concentrations (0 to 450 µM) using KH<sub>2</sub>PO<sub>4</sub> as P source. Growth traits like biomass, leaf area and chlorophyll were recorded on 21 days old seedlings and finally selected sufficient or optimum and low P concentrations (Suppl. Fig. 2). The growth conditions maintained in the Controlled Environment Facility was day/night temperature at 30/27°C, relative humidity of 85%, photoperiod of 12 h at a photosynthetic photon flux density (PPFD) 1000 µmol m<sup>-2</sup> s<sup>-1</sup>.

Drought stress was imposed in two sets of treatment, after a week of transferring the plants to nutrient solution by hanging the roots in the air following method of [41]. Initially, seedling roots were exposed to air for 1 hr for acclimatization after which the exposure time was increased to 3 h. This dehydration treatment was repeated consecutively for a period of five days for the same plant to lower the water potential. The exposure time to air was from 10.00 h to 13.00 in drought treatment. Two weeks post-dehydration treatment, the morpho-physiological parameters were recorded for screening the accessions for combined tolerance to drought and low P stress. Out of 100, 10 accessions were selected based on their performance under combined stress for Experiment 2 in soil culture.

### 2.2 Experiment 2: Evaluation of selected accessions in soil

From the results obtained from Experiment 1, ten mungbean accessions were grown in soil during summer (22 March to 15 June) 2018 to assess the growth, yield, physiological and biochemical basis of low P, drought and combined stress tolerance. Plants were grown under sufficient and low P availability in soil. Initially eight seeds were sown in plastic pots (30 cm diameter) containing 15 kg sandy loam soil. The pH (soil: water, 1:5) and electrical conductivity (EC) of the soil was 7.8 and 0.155 mS m<sup>-1</sup> respectively. Available P [42] was 7.8 mg P kg<sup>-1</sup> soil. The design of experiment was completely randomized design (CRD) with 10 accessions, two P levels and two moisture regimes. For each accession and treatment, ten pots were maintained with two plants per pot, and one pot was treated as one replicate. Recommended rate of nitrogen (20 kg N ha<sup>-1</sup>) and potash (60 kg K<sub>2</sub>O ha<sup>-1</sup>) as urea and muriate of potash, respectively for mungbean were mixed with soil. Single super phosphate was applied at the recommended rate (40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) to create sufficient P level while no extra P was added to low P pots. Fifteen days after sowing, three healthy and uniform plants per pot with ten pots for each treatment were maintained. Drought stress was created by withholding irrigation for 10 days from 35 days after sowing resulting in 10-11% reduction in soil moisture which was maintained for two days, and subsequently plants were re-watered. To assess the physiological and biochemical traits related to drought and P stress, destructive sampling was done from three pots after imposition of drought stress. The remaining seven pots were used for recording yield attributes.

### 2.3 Water relation traits and Membrane stability index (MSI)

Scanning and image analysis for root traits in hydroponically grown 100 accessions was carried out by EPSON scanner and WinRhizo program (Regent Instruments Inc., Canada), respectively. The traits such as total root length (cm plant<sup>-1</sup>), total root surface area (cm<sup>2</sup> plant<sup>-1</sup>), root volume (cm<sup>3</sup> plant<sup>-1</sup>) and root average diameter (cm) were obtained. Chlorophyll concentration was measured by an optical chlorophyll content meter (MC-100, Apogee Instruments, USA) in the second trifoliate leaf of the seedlings and expressed as  $\mu\text{mol}$  chlorophyll m<sup>-2</sup> leaf area.

Rate of water loss (RWL) from leaf was estimated according to [45]

$$\text{RWL (g H}_2\text{O g}^{-1} \text{ dry weight h}^{-1}) = (\text{W}_0 \text{ min} - \text{W}_{140} \text{ min}) / \text{dry wt. (g)} \times \text{time (h)} \quad (1)$$

Moisture retention capacity (MRC) calculated using the following formulae:

$$\text{MRC (\%)} = (\text{weight after 140 min} - \text{dry weight}) / (\text{fresh weight} - \text{dry weight}) \times 100 \quad (2)$$

Excised leaf weight loss (ELWL) after 30, 90, and 140 minutes was calculated as water lost per unit of initial water content in the leaf according to [46] as follows:

$$\text{ELWL}_{\text{W}_0-30 \text{ min}} = (\text{FW}_0 - \text{FW}_{30}) / (\text{FW}_0 - \text{DW}) \quad (3)$$

$$\text{ELWL}_{\text{W}_{30}-90 \text{ min}} = (\text{FW}_{30} - \text{FW}_{90}) / (\text{FW}_{30} - \text{DW}) \quad (4)$$

$$\text{ELWL}_{\text{W}_{90}-140 \text{ min}} = (\text{FW}_{90} - \text{FW}_{140}) / (\text{FW}_{140} - \text{DW}) \quad (5)$$

Where FW is fresh weight and DW is dry weight of leaf tissue

The RWC was estimated according to reference [47], whereas MSI was measured in the fully expanded third trifoliate leaf according to method proposed by reference [48].

### 2.4 Tissue phosphorus concentration

P concentration was estimated in stem, leaf, seed and root using oven dried tissue. The ground samples were digested with di-acid mixture (HNO<sub>3</sub>: HClO<sub>4</sub>, 9:4) and after volume made up to 50 mL, it was filtered through Whatman No. 42. The samples were run on inductively-coupled plasma optical emission spectrometer (ICP-OES; model 5110, Agilent technologies, USA) which was calibrated using standard. The results of four replications were averaged and P concentration was expressed as mg g<sup>-1</sup> dry weight.

### 2.5 Photosynthetic pigment and gas exchange measurements in selected accessions

Chlorophyll and carotenoid content were estimated on fully expanded third trifoliate leaf by non-maceration method using dimethyl sulfoxide [49]. The gas exchange traits were measured using infrared gas analyser (Li-6800, Li-COR Inc., NE, USA) on the second fully opened trifoliate leaf. The measuring conditions of leaf chamber was set at 1200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetic photon flux density, CO<sub>2</sub> concentration set at 400  $\mu\text{mol mol}^{-1}$ , relative humidity at 65±2% and temperature at 28±1°C. The measurements were recorded between 8.00-12.00 hr. Instantaneous water use efficiency (IWUE,  $\mu\text{mol mol}^{-1}$ ) was calculated by the photosynthetic rate divided by transpiration rate.

### 2.6 Characterization and quantification of organic acid in root exudates in selected accessions

After imposition of drought, the plants were uprooted carefully and roots washed with deionised water. The roots were immersed in 50 mL of trap solution (0.5 mM CaCl<sub>2</sub> solution, pH 4.5) contained in 100 mL Erlenmeyer flask wrapped in black paper and kept under natural condition for 4 hours (8.00 to 12.00 h). Root fresh weight was recorded after removing the plants from the trap solution and the quantification of organic acid was carried out following the method of [50]. The root exudate (20 mL) was passed through a cation exchange column (Amberlite IR-120 H<sup>+</sup> form) followed by passing through an anion-exchange column (DEAE-cellulose). The anions were eluted with 2.0 mL of 1.0 M HCl and after drying, the pellet was re-suspended in 500  $\mu\text{L}$  of 5 mM H<sub>2</sub>SO<sub>4</sub>. Samples were analysed using HPLC

(Agilent Technologies, 1200 Infinity) with Hi-Plex H column as the stationary phase. The column temperature was set at 70°C. Mobile phase (5 mM H<sub>2</sub>SO<sub>4</sub>) was used at a flow rate of 0.6 mL min<sup>-1</sup>. Individual samples were run for 25 min and peaks captured by a refractive index detector (RID) with an optical temperature of 55°C. For peak identification, standard solutions of oxalic, citric, pyruvic,  $\alpha$ -ketoglutaric, malic, maleate, malonate, succinic, lactic, acetic and fumaric acids were used (procured from Sigma). Concentration of individual acids were quantified from the calibration curves of standards and expressed as  $\mu\text{mol g}^{-1}$  root fresh weight while total organic was calculated as the sum of individual acids. The relative organic acid was computed by total organic acid exudation under treated conditions divided by total organic acid exudation under control condition.

## 2.7 Grain yield and its attributes in selected accessions

At maturity, pods were harvested in two pickings, viz. at 42 and 55 days after sowing. Total seed yield was determined by summing up both pickings from single plant, weighed and expressed as g plant<sup>-1</sup>. The total number of pods was counted for each plant and expressed as pods per plant. Six pods were randomly selected and threshed, number of seeds were counted and expressed as number of seeds per pod. For 100 seed weight (test weight), seeds were counted manually and weighed.

## 2.8. Stress tolerance evaluation in hydroponics (PCA ranking)

The stress tolerance of accessions under different treatments were evaluated by calculating the relative value which was obtained by the formula:

$$\text{Relative value} = (\text{trait value under treatment}) / (\text{trait value under controlled}) \times 100 \quad (6)$$

The principal component analysis (PCA) ranking value was used for assessing stress tolerance of accessions under different treatments. For each mungbean accession, ranking value was determined by the formula [51]:

$$\text{PCA ranking value} = (\text{contribution of PC1 (\%)} \times \text{PC1}) + (\text{contribution of PC2 (\%)} \times \text{PC2}) + (\text{contribution of PC3 (\%)} \times \text{PC3}) + (\text{contribution of PC4 (\%)} \times \text{PC4}) \quad (7)$$

## 2.9 Statistical analysis

The experiments were laid out in three-factor factorial completely randomised design (CRD) with two P levels, two water regimes and mungbean accessions. For basic statistical calculations, three-way analysis of variance (ANOVA) was carried out in the statistical software R version 3.6.1 [52]. Comparisons among treatments were performed using critical difference (CD) at the 0.05 probability level. All the measured traits were subjected to principal component analysis (PCA) to identify common trend of the multidimensional data sets. PCA was performed using the FactoMineR [53], whereas GraphPad Prism 5.0 and R software were used for data visualization as boxplots and heatmap, respectively.

# 3. Results

## 3.1 Evaluation of mungbean core in hydroponics

The 100 mungbean accessions screened under different treatments in hydroponics culture for traits related to drought stress (excised leaf weight loss, rate of water loss and moisture retention capacity), biomass (dry shoot weight, dry root weight, total dry weight and root to shoot ratio), root morphology (total root length, root surface area, average root diameter, root volume and number of root tips), and P indices (P concentration and total plant P uptake) were evaluated. The effect of various levels of P, moisture regime, and genotypes as well as their interactive effects (P×W, P×G and W×G) were significant ( $P \leq 0.05$ ) for all the parameters except ELWL (Suppl. Table 2). To evaluate the relative contribution of each trait under different treatments, relative values of all traits were subjected to PCA. It was found that under low P stress, PC1 and PC2 explained 39.4 and 22.7% genotypic variation, respectively (Fig. 1a). The traits contributing to this variation under low P stress were total dry weight, shoot dry weight, root dry weight, total root length, root surface area, root volume, moisture retention capacity, rate of water loss, number of root tips and total plant P uptake. Under drought stress, PC1

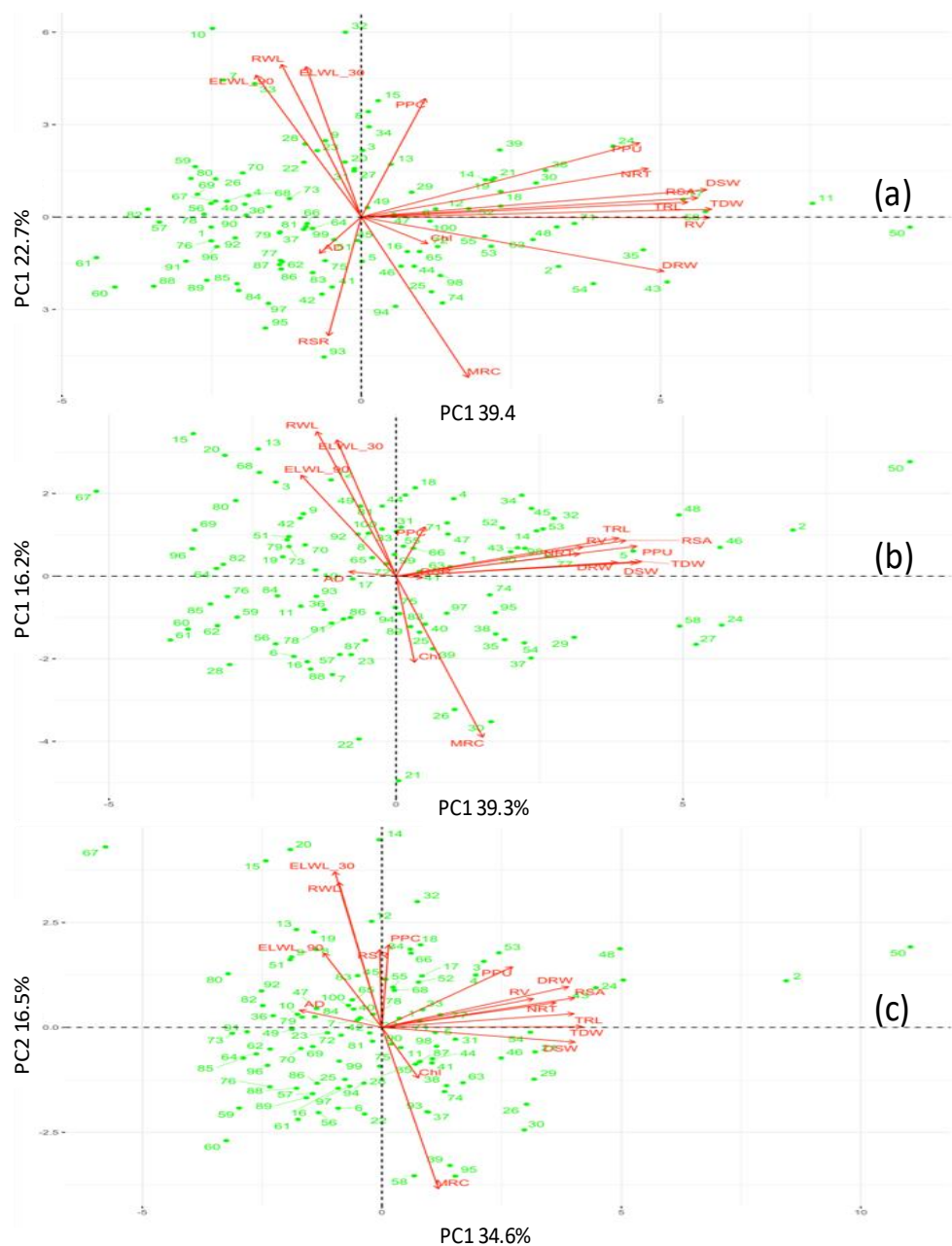


and PC2 explained 39.3 and 16.2% genotypic variation respectively (Fig. 1b). The major contributing traits under drought stress were total dry weight, shoot dry weight, root surface area, total root length, total plant P uptake, moisture retention capacity and rate of water loss. Under combined stress, the PC1 and PC2 explained 34.6% and 16.5% phenotypic variation, respectively (Fig. 1c). The most contributing traits to the genetic variation under combined stress were found as total dry weight, shoot and root dry weight, root surface area, total root length, total plant P uptake, moisture retention capacity and excised leaf weight loss. However, the contributing traits common between three stress treatments were the same as those mentioned for drought stress except for rate of water loss.

### 3.2 Differential response of contrasting groups to low P, drought and combined stress

The PCA ranking values were calculated using loading scores from the relative values of all traits under low P, drought and combined stresses (Supplementary Table 3). Based on the PCA ranking values, top 25 accessions with higher rank values were grouped as tolerant and 25 accessions from bottom with low rank values were grouped as sensitive to drought, low P and combined stress (Table 1). The tolerant accessions under all three treatments exhibited higher relative values in comparison to the sensitive ones. In the tolerant group, the average of shoot biomass was reduced by 25, 61 and 76% while in the sensitive group, the reduction was 62, 79 and 82% respectively under low P, drought and combined stress in comparison to control (Fig. 2a). Similarly, the mean of total biomass in tolerant group was reduced by 14, 58 and 73% whereas in sensitive group, it decreased by 55, 78 and 81% under low P, drought and combined stress respectively as compared to control (Fig. 2b). Increase in chlorophyll concentration, a low P stress specific trait, was observed in tolerant group while it decreased under drought in both groups (Fig. 2c).

The total root length decreased drastically in both groups in all treatments except in tolerant group at low P which was non-significant (Fig. 2d). Under drought and combined stresses, more than 90% reduction was noted in group mean for total root length as compared to control. The mean of root surface area in the tolerant group increased under low P while it decreased in the sensitive group (Fig. 2e). Likewise, root surface area also decreased (>80%) under drought and combined stress as compared to control. The average root diameter in both tolerant and sensitive groups increased markedly under drought and combined stress while under low P, less than 15% increase was noted indicating root thickening under drought and combined stress (Fig. 2f). The average for root-to-shoot ratio increased in both tolerant and sensitive groups under all treatments as compared to control, maximum being in sensitive group under low P (Fig. 2g). A reduction in total P uptake in both groups was observed but it was drastically reduced in sensitive group compared to tolerant group (Fig. 2h). On an average, the rate of water loss from leaves was higher in both groups under low P stress as compared to control (Fig. 2i).



**Figure 1.** Biplot using relative values of 16 physiological traits of mungbean accessions grown hydroponically under (a) low phosphorus, (b) drought, and (c) combined stress. Arrow represents agro-physiological traits, its length corresponds to the contribution of each trait. Number represents 100 mung bean accessions corresponding to Supplementary Table 1. Abbreviation: ELWL\_30 and ELWL\_90, excised leaf water loss per unit initial water content after 30 minutes and 90 minutes; RWL, rate of water loss; MRC, moisture retention capacity; PPC, plant P concentration; PPU, plant P uptake; Chl, chlorophyll concentration; DSW, dry shoot weight; DRW, dry root weight; TDW, total dry weight; RSR, root-to-shoot ratio; TRL, total root length; RSA, root surface area; AD, Average root diameter; RV, root volume; NRT, number of root tips

**Table 1.** Selection of tolerant and sensitive mungbean accessions (25 each) based on their response to different stresses viz., low P, drought and combined (low P and drought). These accessions were

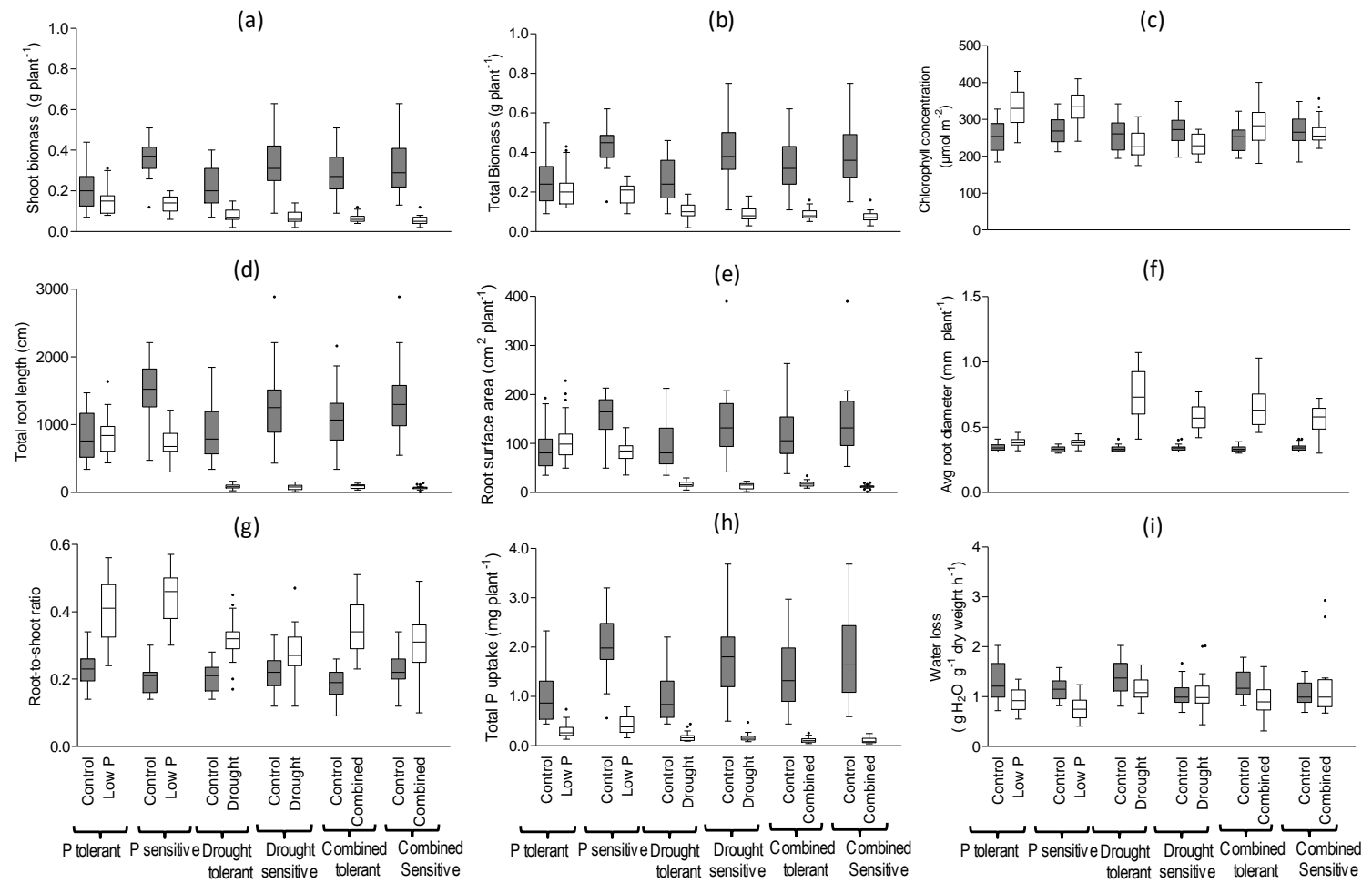
identified on the basis of principal component analysis ranking using loading score from relative values under treated conditions. The ranking values are presented in Supplementary Table 3.

Low P		Drought		Combined	
Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive
IC314606	EC398916	EC398916	EC398907	<b>IC280489</b>	EC398907
EC396156	EC398414	IC314606	IC565301	IC76468	EC396156
IC257571	IC252008	IC507276	IC73112	IC489015	IC489004
IC401586	IC305250	IC507418	IC76370	IC76414	IC148401
IC305222	IC507526	IC257571	IC357035	IC507321	IC565301
IC565301	IC76422	IC401586	IC305250	<b>EC397142</b>	IC73112
IC148442	IC488849	IC305222	IC507526	EC397141	EC398131
<b>IC280489</b>	IC488808	IC148442	IC488582	IC76466	IC488904
EC398131	IC118993	<b>IC280489</b>	IC76346	IC76499	IC507504
IC76468	<b>IC119005</b>	IC507527	IC398746	<b>IC76415</b>	IC76451
IC507527	IC405261	IC76414	IC488808	<b>IC333090</b>	IC488582
<b>EC397142</b>	<b>IC73401</b>	IC507321	IC118993	<b>IC507340</b>	IC76346
EC397141	EC396413	<b>EC397142</b>	EC398880	IC76422	IC489093
IC285532	IC488875	EC397141	<b>IC119005</b>	IC488808	IC121249
IC488813	<b>IC488526</b>	IC507320	IC754464	EC398937	EC398880
IC507504	IC119018	<b>IC76415</b>	<b>IC73401</b>	EC398886	<b>IC119005</b>
<b>IC76415</b>	<b>IC325853</b>	IC507483	EC314285	IC405261	<b>IC73401</b>
<b>IC333090</b>	IC76581	<b>IC333090</b>	<b>IC488526</b>	IC305249	EC314285
<b>IC507340</b>	IC507296	<b>IC507340</b>	IC119018	<b>IC121316</b>	<b>IC488526</b>
IC507376	IC590082	IC76422	<b>IC325853</b>	EC396413	<b>IC325853</b>
IC398746	IC623704	IC488966	IC553566	IC315045	IC507296
IC282110	IC623821	EC398949	IC507296	IC623821	IC590082
IC488966	IC584699	IC314512	IC590082	IC573083	IC623704
EC398949	IC415097	<b>IC121316</b>	IC623821	IC623705	IC415097
IC305249	HUM-1	IC507319	IC393792	IC512343	IC393792

**Bold** text represents the tolerant accessions which were common under all three treatments while ***bold italics*** text represents the sensitive accession which were common under all three treatments.

From Table 1, we selected those accessions which were common in all three stress treatments as tolerant and sensitive (Table 1). So, based on PCA rankings we found six tolerant (IC280489, EC397142, IC76415, IC333090, IC507340, and IC121316) and four (IC119005, IC73401, IC488526, and IC325853) sensitive accessions from the hydroponics experiment which were used for detailed physiological studies in soil culture.





**Figure 2.** Variation among contrasting group of mungbean genotypes with respect to (a) shoot biomass, (b) total biomass, (c) chlorophyll concentration, (d) total root length, (e) root surface area, (f) root average diameter, (g) root-to-shoot ratio, (h) total P uptake, (i) rate of water loss at control, low P, drought and combined stress conditions. Black line (–) inside boxes indicates median, plus symbol (+) denotes mean, box hinges indicate first and third quartiles, and whiskers indicate full range of the data. Solid circles denote outliers according to Tukey's test.

### 3.3 Stress susceptibility index of 10 mungbean accessions grown in soil culture

The selected ten accessions from Experiment I were grown in soil under different treatment combinations. We calculated the stress susceptibility index (SSI) for these accessions taking into consideration the above ground dry matter and seed yield in control and low P soil with and without drought stress (Table 2). The ranking was done using online toolkit iPASTIC (<https://manzik.com/ipastic/>). Based on mean ranking values, these accessions were grouped as tolerant (IC333090, IC507340, IC73401, IC121316, IC76415) and sensitive (IC280489, EC397142, IC119005, IC325853, IC488526). There was a slight change in the groupings of tolerant and sensitive accessions according to SSI as against PCA ranking. However, we followed the tolerant and sensitive groupings derived from SSI values to present the various physiological responses to stress treatments in soil culture.

**Table 2.** Stress susceptibility indices (SSI) of 10 selected mungbean accessions based on the above ground dry matter (AGDM) and seed yield. Rank 1 is the best performer, while rank 10 is worst performer.

	Low P		Drought		Combined		Mean
	AGDM	Seed yield	AGDM	Seed yield	AGDM	Seed yield	
IC333090	1	1	1	1	1	2	1.2
IC507340	6	2	2	2	2	1	2.5
IC73401	3	4	3	4	7	4	4.2
IC121316	5	3	4	5	6	7	5.0
IC76415	2	7	7	7	4	5	5.3
IC280489	4	8	8	6	5	3	5.7
EC397142	7	5	9	3	8	6	6.3
IC119005	10	10	6	8	3	8	7.5
IC325853	8	6	5	9	9	9	7.7
IC488526	9	9	10	10	10	10	9.7

### 3.4 Biomass, RWC and MSI influenced by low P, drought and combined stress

Significant ( $P \leq 0.05$ ) influence of P levels, drought and accessions were observed on RWC, MSI and dry matter accumulation but  $P \times W$  interaction was not significant for MSI (Suppl. Table 4). Averaged over accessions, the above ground dry matter decreased by 16.4, 24.7 and 32.3% in tolerant group, whereas in sensitive group, the reduction was 27.4, 33.0 and 48.6% under low P, drought and combined stress, respectively as compared to control (Fig. 3a). Marked reduction in dry matter accumulation was observed in accession IC488526 followed by IC325853 whereas, least reduction was noted in accession IC333090 followed by IC507340 under combined stress.

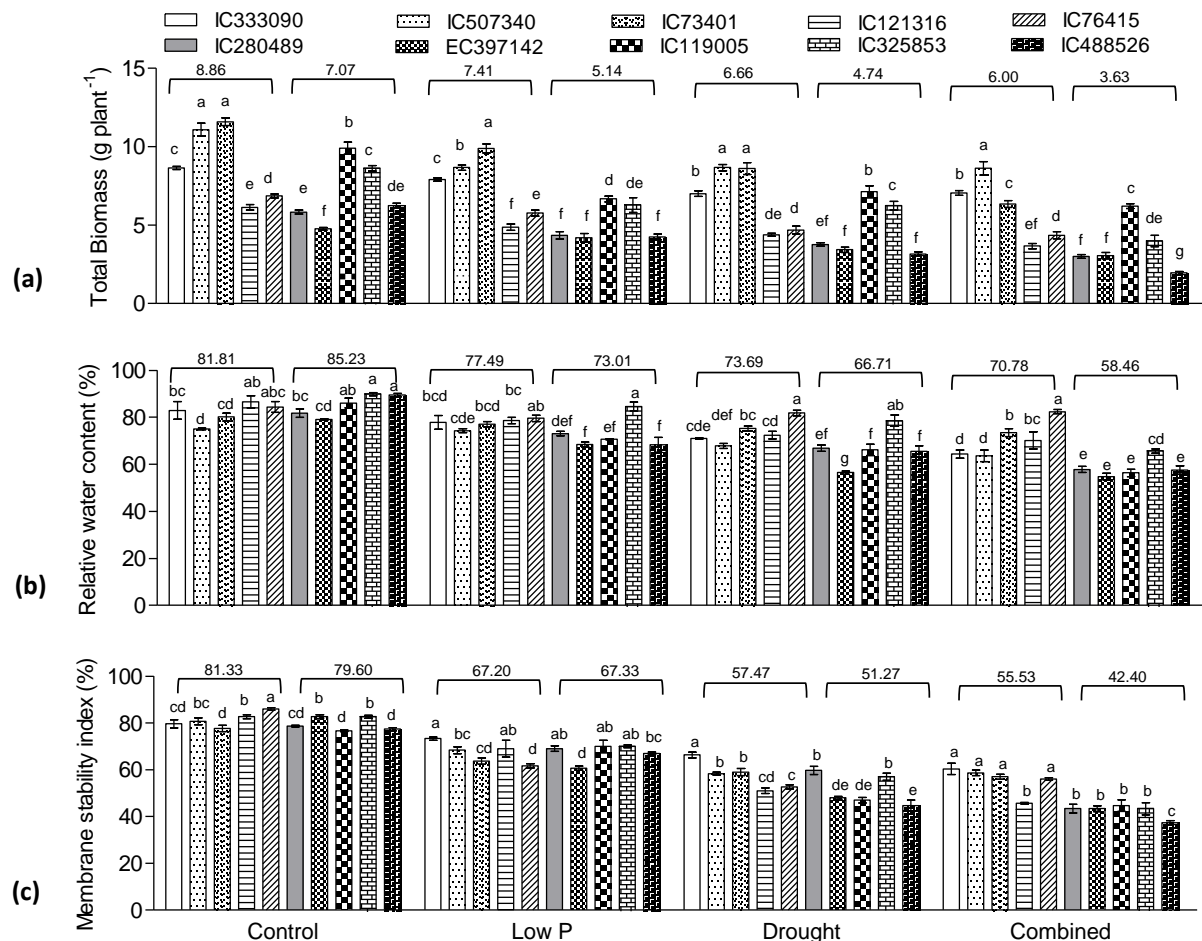
Under combined stress, lesser reduction in RWC (13.5%) and MSI (32.9%) was found in tolerant group as compared to sensitive group (RWC 22% and MSI 41.6%) (Fig. 3b,c). Further, among the tolerant accessions, the least reduction in RWC was recorded in accessions IC76415 and IC73401 under combined stress. Similarly, the MSI was least affected in IC333090 and IC507340 among the tolerant group while accessions IC488526 and IC280489 were highly influenced by combined stress.

### 3.5 Effect of low P, drought and combined stress on phosphorus indices

Though low P and drought significantly reduced the P concentration in shoot and seed but a drastic effect was observed in shoot tissue P concentration under combined stress (Suppl. Table 4; Fig. 4a,c). The reduction in P concentration in seed and shoot in IC333090 was non-significant whereas it was highest (31 to 40%) in IC488526 under combined stress as compared to control. The P uptake in

seed and shoot were significantly reduced in all treatments (Fig. 4b,d). Among treatments, the maximum reduction in shoot P uptake was noted in combined stress as compared to low P and drought (Fig. 4b). Further, the accessions in sensitive group showed higher reduction than the tolerant group. Similarly, the seed P uptake in tolerant group was reduced by 30, 41 and 53% whereas it was 32, 63 and 77% in sensitive group under low P, drought and combined stress, respectively as compared to control (Fig. 4d). Among accessions, highest reduction in P uptake in seed and shoot was noted in IC488526 (sensitive) under individual or combined stresses while IC333090 and IC507340 (tolerant) showed least reduction.

The P harvest index (PHI) decreased significantly in all treatments with highest reduction noted in drought stress as compared to control (Fig. 5a). Among tolerant and sensitive groups, the PHI decreased by 37 and 17% respectively under combined stress in comparison to control. The PUEg (PUE for grain) averaged over accessions was higher under low P stress while it decreased under drought and combined stress (Fig. 5b). This reduction was higher in sensitive rather than the tolerant group. More than 50% decrease in PUEg under combined stress was noted in accession IC488526. Conversely, the PUEbm (PUE for biomass) was higher in all three treatments as compared to control (Fig. 5c). Between treatments, low P stress resulted in higher PUEbm as compared to control while among accessions, the PUEbm was highest in IC488526 (55%) and EC397142 (44%) and least in IC333090 and IC507340.

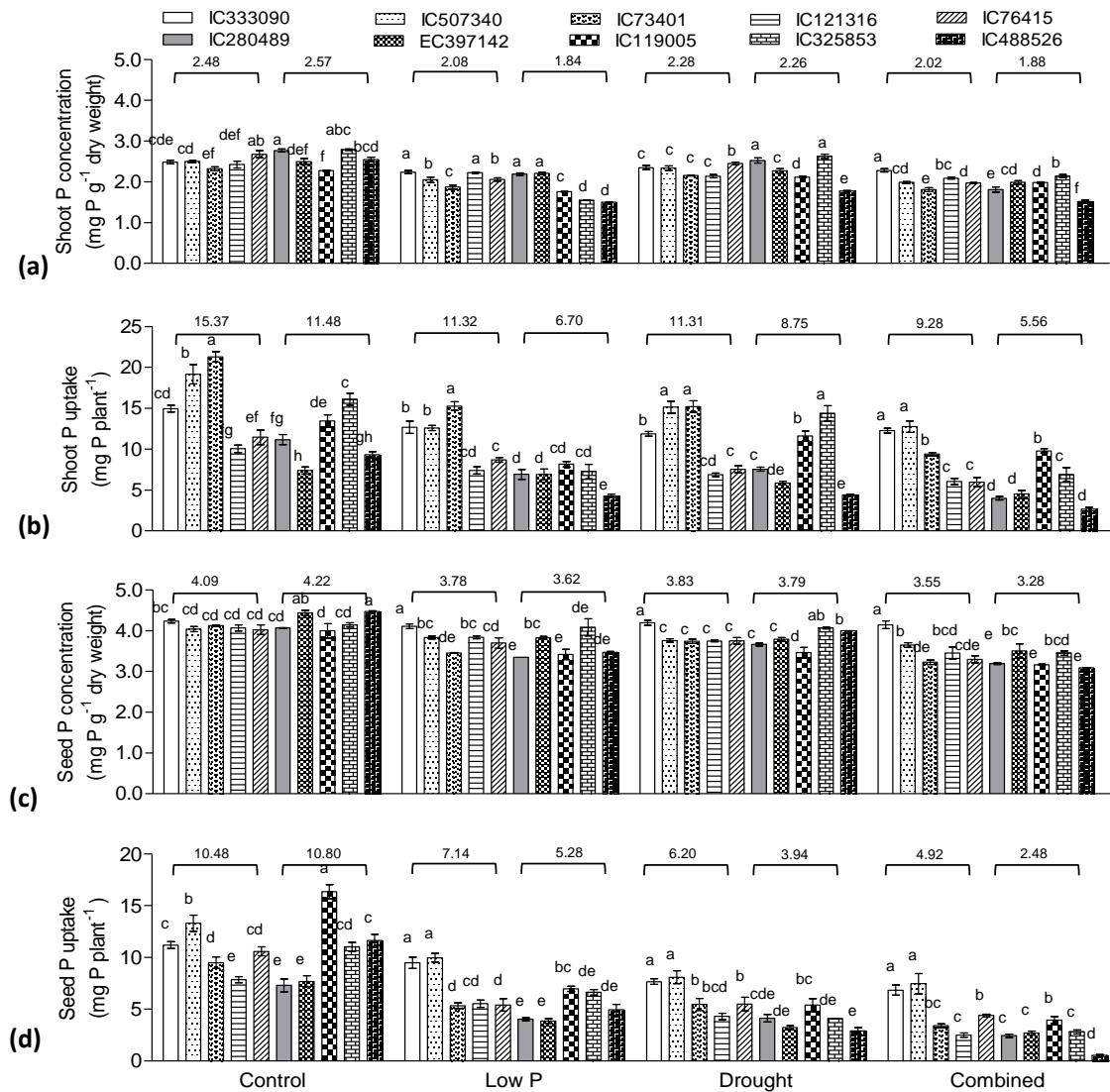


**Figure 3.** Effect of low P, drought and combined stress on (a) total above ground dry matter, (b) relative water content, and (c) membrane stability index in 10 mung bean accessions. Values on bars 1-5 and 6-10 represents mean of tolerant and sensitive accessions respectively. The letters on each bar denotes significant difference between accessions in each treatment. Error bars indicate mean  $\pm$  SEM.

### 3.6 Gas exchange traits and photosynthetic pigments influenced by drought and low P stress

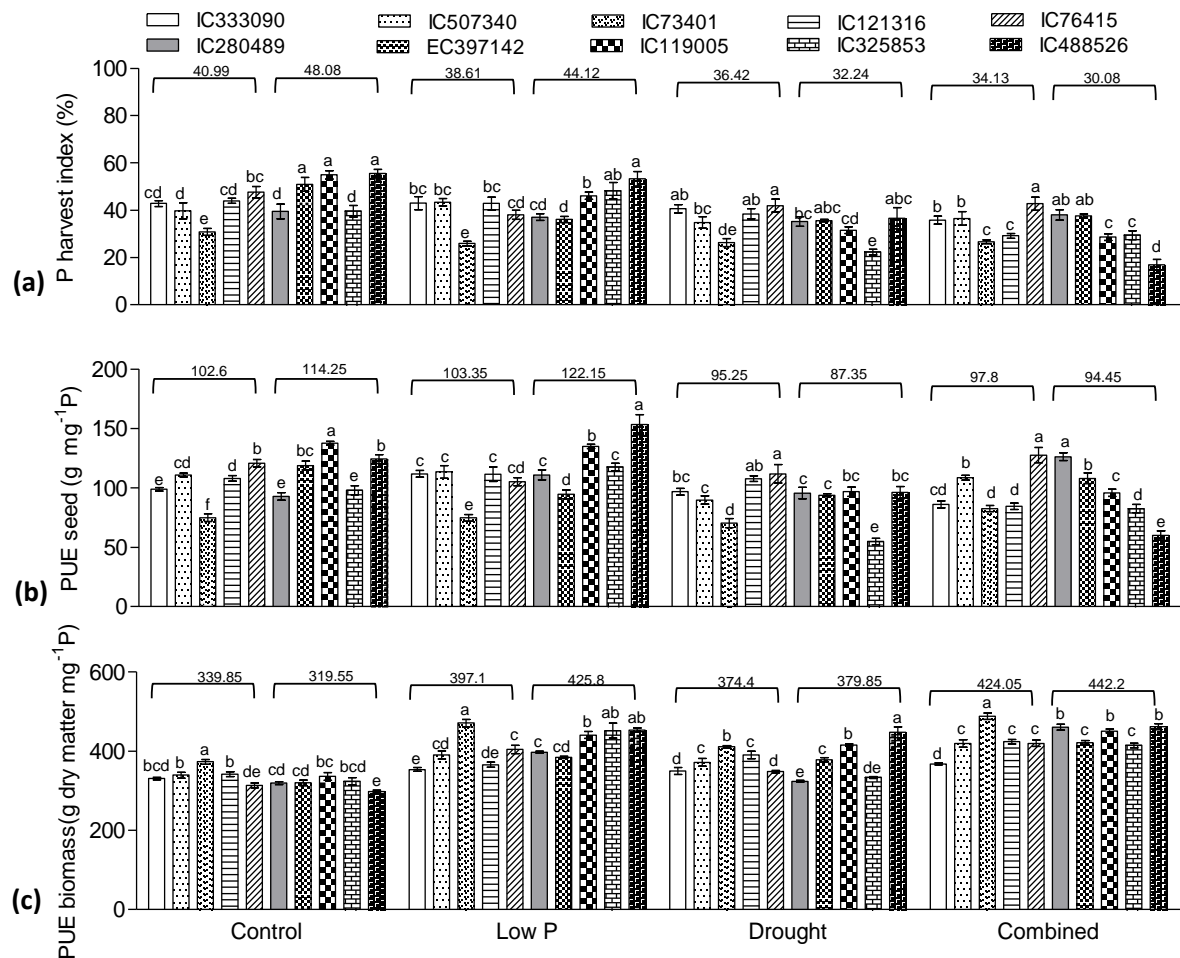
The gas exchange traits were significantly ( $P \leq 0.05$ ) affected by P, W, G, as well as their interactions ( $P \times W$ ,  $P \times G$ ,  $W \times G$  and  $P \times W \times G$ ) except for  $P \times W$  effect on transpiration (Suppl. Table 5). Drought and low P stress showed an adverse effect while the combined stress drastically reduced photosynthesis ( $P_N$ ), transpiration (E) and stomatal conductance ( $g_s$ ) (Fig. 6a-c). However, recovery from drought and combined stress resulted in a significant increase in  $P_N$ , E and  $g_s$ , the recovery being higher in drought as compared to combined stress. The reduction in  $P_N$  due to combined stress in sensitive group was between 95-98% while it ranged between 71-92% in tolerant group (Fig. 6a). Similarly, the rate of transpiration under low P, drought and combined stress significantly decreased by 13, 70 and 83% in tolerant group, and by 19, 73 and 92% in sensitive group, respectively (Fig. 6b). The response pattern for  $g_s$  in all accessions was similar to E (Fig. 6c). On the other hand, the IWUE increased under drought and combined stress in comparison to control (Fig. 6d). Further, recovery from drought and combined stress resulted in increased IWUE as compared to control, which was maximum in sensitive group as compared to that in tolerant group particularly under recovery from combined stress. Among accessions, IC333090 showed tolerance whereas IC488526 exhibited drastic reduction in gas exchange traits in response to drought, low P and combined stresses.

Total chlorophyll and carotenoid concentration in leaves were significantly influenced by P, moisture regimes and accessions as well as their interactions. However, the effect of P and  $P \times W$  interaction was not significant for total chlorophyll (Suppl. Table 5; Suppl. Fig. 3a,b). Averaged over accessions, total chlorophyll was higher in tolerant group under combined stress as compared to control. The value averaged over accessions for carotenoid showed increased concentration by 27.9 and 16.4% in tolerant and sensitive groups, respectively under combined stress.

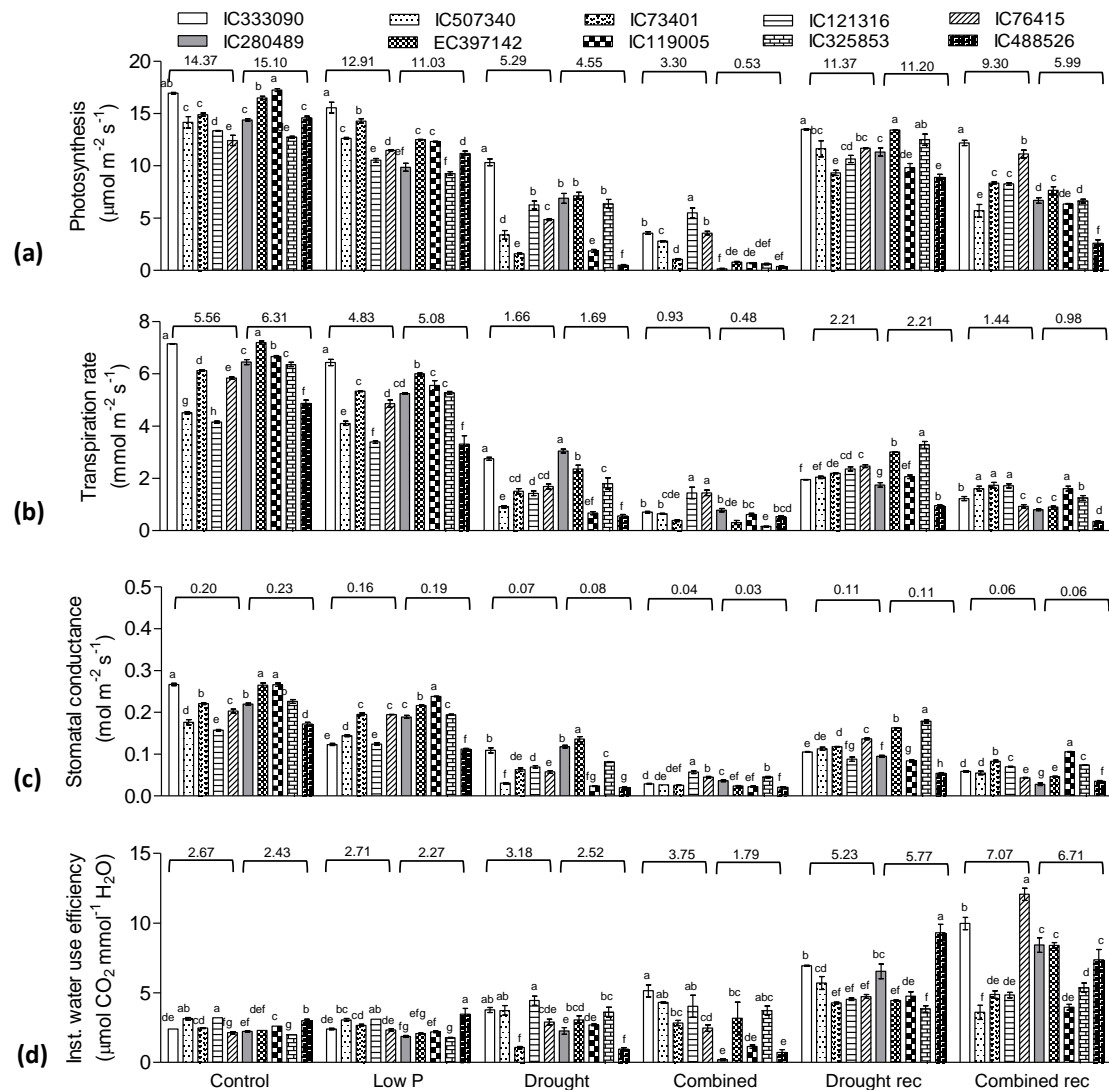


**Figure 4.** Effect of low P, drought and combined stress on (a) shoot P concentration, (b) seed P concentration, (c) shoot P uptake, and (d) seed P uptake in 10 mung bean accessions. Values on bars 1-5 and 6-10 represents mean of tolerant and sensitive accessions respectively. The letters on each bar denotes significant difference between accessions in each treatment. Error bars indicate mean  $\pm$  SEM.





**Figure 5.** Effect of low P, drought and combined stress on (a) P harvest index (%), (b) PUEg (g seed  $\text{mg}^{-1}$  P), (c) PUEbm (g dry matter  $\text{mg}^{-1}$  P) in 10 mung bean accessions. Values on bars 1-5 and 6-10 represents mean of tolerant and sensitive accessions respectively. The letters on each bar denotes significant difference between accessions in each treatment. Error bars indicate mean  $\pm$  SEM.



**Figure 6.** Effect of low P, drought and combined stress on (a) rate of photosynthesis, (b) transpiration, (c) stomatal conductance, and (d) instantaneous water use efficiency in 10 mung bean accessions. Values on bars 1-5 and 6-10 represents mean of tolerant and sensitive accessions respectively. The letters on each bar denotes significant difference between accessions in each treatment. Error bars indicate mean  $\pm$  SEM.

### 3.7 Organic acid exudation in response to low P, drought and combined stress

The quantity of various organic acids in root exudate was significantly ( $P \leq 0.05$ ) influenced by P, W, and G, as well as their interaction effects (Supplementary Table 6; Tables 3a-c). In general, exudation of organic acid was higher under low P as compared to control except for pyruvate, which showed reduction under all treatments. Interestingly, no additive effect of drought under low P stress was found on total organic acid exudation in either groups. Oxalate was not detected in control and drought but low P stress showed considerable exudation. However, combined stress induced significantly higher quantity of oxalate but the accessions exhibited differential response. Similarly, citrate was induced under low P and combined stress but there was differential response of accessions under drought as four out of ten accessions exuded remarkably higher quantity of citrate.

Under low P stress, exudation of maleate (4.8-fold), lactate (3.4-fold),  $\alpha$ -ketoglutarate (2.9-fold), fumarate (1.8-fold) and succinate (1.3-fold) increased in tolerant as compared to sensitive group but the quantity of acetate and malonate was higher in sensitive group. Likewise, under drought stress, the

amount of maleate (6.5-fold), lactate (3.4-fold), succinate (2.7-fold), malate (2.2-fold) and fumarate (1.4-fold) increased in tolerant as compared to sensitive group. Among these accessions, low P and drought stress induced maximum quantity of total organic acid in IC73401 while under combined stress, it was maximum in IC333090 which was higher than the average of tolerant group. Likewise, IC325853 exuded consistently increased amount of total organic acid under all treatments which was higher than the average of sensitive group.

**Table 3a.** Effect of P nutrition and drought on oxalate, citrate, maleate and  $\alpha$ -ketoglutarate content in root exudation of selected mungbean accessions grown at two P levels (sufficient, 25.6 mg P kg<sup>-1</sup> dry soil, and low P, 7.8 mg P kg<sup>-1</sup> dry soil) and two moisture regimes (irrigated and drought).

Genotype	Oxalate ( $\mu$ M per g <sup>-1</sup> root FW)				Citrate ( $\mu$ M per g <sup>-1</sup> root FW)			
	Control	Low P	Drought	Combined	Control	Low P	Drought	Combined
IC333090	nd	6.11	nd	nd	nd	17.5	nd	1.69
IC507340	nd	2.71	nd	4.64	nd	0.92	nd	2.77
IC73401	nd	nd	nd	nd	nd	0.91	48.0	4.01
IC121316	nd	11.1	nd	nd	nd	25.0	nd	0.42
IC76415	nd	6.43	nd	9.85	nd	14.2	47.5	0.54
<b>Mean (Tolerant)</b>	--	<b>6.59</b>	--	<b>7.25</b>	--	<b>11.7</b>	<b>47.8</b>	<b>1.89</b>
IC280489	nd	nd	nd	nd	nd	7.68	nd	1.07
EC397142	nd	16.5	nd	20.2	nd	1.33	54.1	2.56
IC119005	nd	8.57	nd	nd	nd	5.60	nd	0.22
IC325853	nd	4.29	nd	nd	nd	7.85	nd	7.89
IC488526	nd	5.42	nd	nd	nd	2.24	34.4	0.74
<b>Mean (Sensitive)</b>	--	<b>8.70</b>	--	<b>20.2</b>	--	<b>4.94</b>	<b>44.3</b>	<b>2.50</b>
CD (5%) value		P	W	G	P×W	P×G	W×G	P×W×G
Oxalate		0.37	0.37	0.83	0.53	1.18	1.18	1.67
Citrate		0.52	0.52	1.17	0.74	1.65	1.65	2.34
Genotype	Maleate ( $\mu$ M per g <sup>-1</sup> root FW)				$\alpha$ -Ketoglutarate ( $\mu$ M per g <sup>-1</sup> FW)			
	Control	Low P	Drought	Combined	Control	Low P	Drought	Combined
IC333090	nd	13.8	5.0	25.3	1.94	117.2	2.09	36.2
IC507340	0.27	171.1	27.4	2.88	2.07	73.8	5.75	9.89
IC73401	0.40	137	nd	8.19	14.0	54.2	5.13	3.75
IC121316	nd	58.2	14.0	1.85	nd	36.9	14.9	2.56
IC76415	nd	20.4	245	0.37	8.65	33.4	0.13	0.65
<b>Mean (Tolerant)</b>	<b>0.34</b>	<b>80.1</b>	<b>72.9</b>	<b>7.72</b>	<b>6.67</b>	<b>63.1</b>	<b>5.6</b>	<b>10.6</b>
IC280489	4.14	40.3	3.50	4.34	8.91	16.3	12.8	2.47
EC397142	0.02	15.1	31.4	3.75	8.83	12.4	22.9	nd
IC119005	nd	18.2	8.43	0.26	28.2	26.3	15.7	0.64
IC325853	7.93	8.12	5.56	23.9	6.60	17.4	6.96	0.22
IC488526	nd	1.81	7.56	6.67	17.3	37.5	6.45	5.31
<b>Mean (Sensitive)</b>	<b>4.03</b>	<b>16.7</b>	<b>11.3</b>	<b>7.78</b>	<b>14.0</b>	<b>22.0</b>	<b>13.0</b>	<b>2.16</b>
CD (5%) value		P	W	G	P×W	P×G	W×G	P×W×G
Maleate		2.86	2.86	6.39	4.04	9.04	9.04	12.8
Ketoglutarate		0.99	0.99	2.21	1.40	3.10	3.10	4.43

'nd' - not detectable; P - phosphorus; W - water; G - genotypes; CD - critical difference.

**Table 3b.** Effect of P nutrition and drought on pyruvate, malonate, malate and succinate content in root exudation of selected mungbean accessions (tolerant and sensitive group) grown at two P levels (sufficient, 25.6 mg P kg<sup>-1</sup> dry soil, and low P, 7.8 mg P kg<sup>-1</sup> dry soil) and two moisture regimes (irrigated and drought).

Genotype	Pyruvate (μM per g <sup>-1</sup> root FW)				Malonate (μM per g <sup>-1</sup> root FW)			
	Control	Low P	Drought	Combined	Control	Low P	Drought	Combined
IC333090	199.6	21.4	6.11	60.8	3.98	10.7	0.46	6.49
IC507340	25.9	3.5	7.24	2.49	2.70	5.76	1.56	3.54
IC73401	10.3	26.3	8.53	60.8	3.03	16.0	2.44	0.29
IC121316	7.27	3.08	17.3	53.3	0.18	5.12	0.90	11.9
IC76415	212	3.16	40.2	30.2	7.26	8.47	14.5	4.23
<b>Mean (Tolerant)</b>	<b>91.0</b>	<b>11.5</b>	<b>15.9</b>	<b>41.5</b>	<b>3.43</b>	<b>9.21</b>	<b>3.98</b>	<b>5.29</b>
IC280489	125.7	2.40	8.92	1.33	11.9	2.36	0.89	5.29
EC397142	230.6	41.5	8.47	46.9	6.33	21.7	6.19	2.85
IC119005	152.0	59.5	21.1	3.80	5.18	4.28	3.76	1.73
IC325853	212.1	69.6	55.8	73.5	16.5	12.5	4.23	24.9
IC488526	4.13	27.9	4.00	46.7	1.44	11.4	1.65	15.0
<b>Mean (Sensitive)</b>	<b>144.9</b>	<b>40.2</b>	<b>19.7</b>	<b>34.5</b>	<b>8.3</b>	<b>10.4</b>	<b>3.3</b>	<b>10.0</b>
CD (5%) value		P	W	G	P×W	P×G	W×G	P×W×G
Pyruvate		2.21	2.21	4.95	3.13	7.00	7.00	9.89
Malonate		0.55	0.55	1.23	0.78	1.74	1.74	2.47
Genotype	Malate (μM per g <sup>-1</sup> root FW)				Succinate (μM per g <sup>-1</sup> root FW)			
	Control	Low P	Drought	Combined	Control	Low P	Drought	Combined
IC333090	1.71	22.5	2.94	3.22	4.55	14.9	0.33	12.2
IC507340	1.52	28.5	0.52	3.58	0.19	13.9	0.26	8.59
IC73401	4.13	1.03	8.60	7.88	1.13	21.0	9.89	12.3
IC121316	0.70	0.60	7.33	5.15	0.68	19.5	19.2	4.95
IC76415	6.01	23.7	3.62	4.60	2.07	45.3	1.42	17.3
<b>Mean (Tolerant)</b>	<b>2.81</b>	<b>15.3</b>	<b>4.60</b>	<b>4.89</b>	<b>1.72</b>	<b>22.9</b>	<b>6.22</b>	<b>11.1</b>
IC280489	2.03	17.2	2.51	4.75	1.82	8.40	0.01	1.61
EC397142	1.49	1.11	0.51	4.20	0.17	7.16	0.42	16.3
IC119005	3.15	51.0	4.32	2.76	0.54	7.71	0.52	2.41
IC325853	2.69	0.73	1.95	11.1	1.39	26.9	2.75	22.4
IC488526	5.66	0.11	1.06	9.09	5.09	35.3	7.62	18.7
<b>Mean (Sensitive)</b>	<b>3.00</b>	<b>14.0</b>	<b>2.07</b>	<b>6.38</b>	<b>1.80</b>	<b>17.1</b>	<b>2.26</b>	<b>12.3</b>
CD (5%) value		P	W	G	P×W	P×G	W×G	P×W×G
Malate		0.59	0.59	1.32	0.84	1.87	1.87	2.65
Succinate		1.06	1.06	2.37	1.50	3.36	3.36	4.75

P – phosphorus; W – water; G – genotypes; CD – critical difference.

**Table 3c.** Effect of P nutrition and drought on lactate, acetate, fumarate and total organic acid content in root exudation of selected mungbean accessions (tolerant and sensitive group) grown at two P levels (sufficient, 25.6 mg P kg<sup>-1</sup> dry soil, and low P, 7.8 mg P kg<sup>-1</sup> dry soil) and two moisture regimes (irrigated and drought). Data correspond to mean of 10 accessions.

Genotype	Lactate (μM per g <sup>-1</sup> root FW)				Acetate (μM per g <sup>-1</sup> root FW)			
	Control	Low P	Drought	Combined	Control	Low P	Drought	Combined
IC333090	1.18	4.94	23.0	2.30	14.5	81.5	7.04	17.4
IC507340	3.71	197.3	18.5	3.93	6.88	25.3	228.5	8.51
IC73401	3.39	252.2	81.7	0.53	3.00	50.9	110.2	4.66
IC121316	17.3	79.5	0.32	34.5	1.36	5.41	62.3	13.6
IC76415	1.46	66.0	35.6	1.44	20.1	2.80	90.6	11.5
<b>Mean (Tolerant)</b>	<b>5.41</b>	<b>120</b>	<b>31.8</b>	<b>8.54</b>	<b>9.16</b>	<b>33.2</b>	<b>99.7</b>	<b>11.1</b>
IC280489	0.47	6.12	23.7	22.0	94.2	50.5	53.2	9.09
EC397142	4.41	116.4	23.5	0.52	5.43	63.0	32.3	0.75
IC119005	3.04	13.2	54.1	15.2	1.35	22.6	162.1	4.56
IC325853	7.26	37.1	31.8	12.7	12.3	92.8	111.0	3.91
IC488526	1.65	0.71	60.6	4.21	9.77	7.61	165.0	24.6
<b>Mean (Sensitive)</b>	<b>3.37</b>	<b>34.7</b>	<b>38.7</b>	<b>10.9</b>	<b>24.6</b>	<b>47.3</b>	<b>104.7</b>	<b>8.6</b>
CD (5%) value		P	W	G	P×W	P×G	W×G	P×W×G
Lactate		5.43	5.43	12.2	7.68	17.1	17.2	24.3
Acetate		2.92	2.92	6.53	4.13	9.24	9.24	13.1
Genotype	Fumarate (μM per g <sup>-1</sup> root FW)				Total organic acid (μM per g <sup>-1</sup> root FW)			
	Control	Low P	Drought	Combined	Control	Low P	Drought	Combined
IC333090	0.40	22.5	75.2	35.4	227.9	333.0	122.2	201.1
IC507340	2.73	66.8	7.81	1.43	46.0	589.5	297.4	52.2
IC73401	3.74	78.3	78.6	0.55	43.1	637.8	353.0	102.9
IC121316	1.64	75.5	58.7	2.75	29.1	319.9	195.0	130.9
IC76415	2.95	43.3	54.9	3.18	260.5	267.2	533.7	83.9
<b>Mean (Tolerant)</b>	<b>2.29</b>	<b>57.3</b>	<b>55.0</b>	<b>8.66</b>	<b>121.3</b>	<b>429.5</b>	<b>300.3</b>	<b>114.2</b>
IC280489	0.21	19.4	30.2	1.93	249.3	170.7	135.8	53.9
EC397142	1.62	41.4	0.09	2.41	258.9	337.6	179.9	100.5
IC119005	19.2	16.5	34.0	1.60	212.5	233.4	304.0	33.1
IC325853	4.29	35.3	70.4	2.10	271.2	312.6	290.5	182.6
IC488526	1.94	39.8	61.6	2.20	47.0	169.7	349.9	133.3
<b>Mean (Sensitive)</b>	<b>5.44</b>	<b>30.5</b>	<b>39.3</b>	<b>2.05</b>	<b>208</b>	<b>245</b>	<b>252</b>	<b>101</b>
CD (5%) value		P	W	G	P×W	P×G	W×G	P×W×G
Fumarate		2.19	2.19	4.89	3.09	6.90	6.90	9.77
Total organic acid		7.09	7.09	15.8	10.0	22.4	22.4	31.7

P – phosphorus; W – water; G – genotypes; CD – critical difference

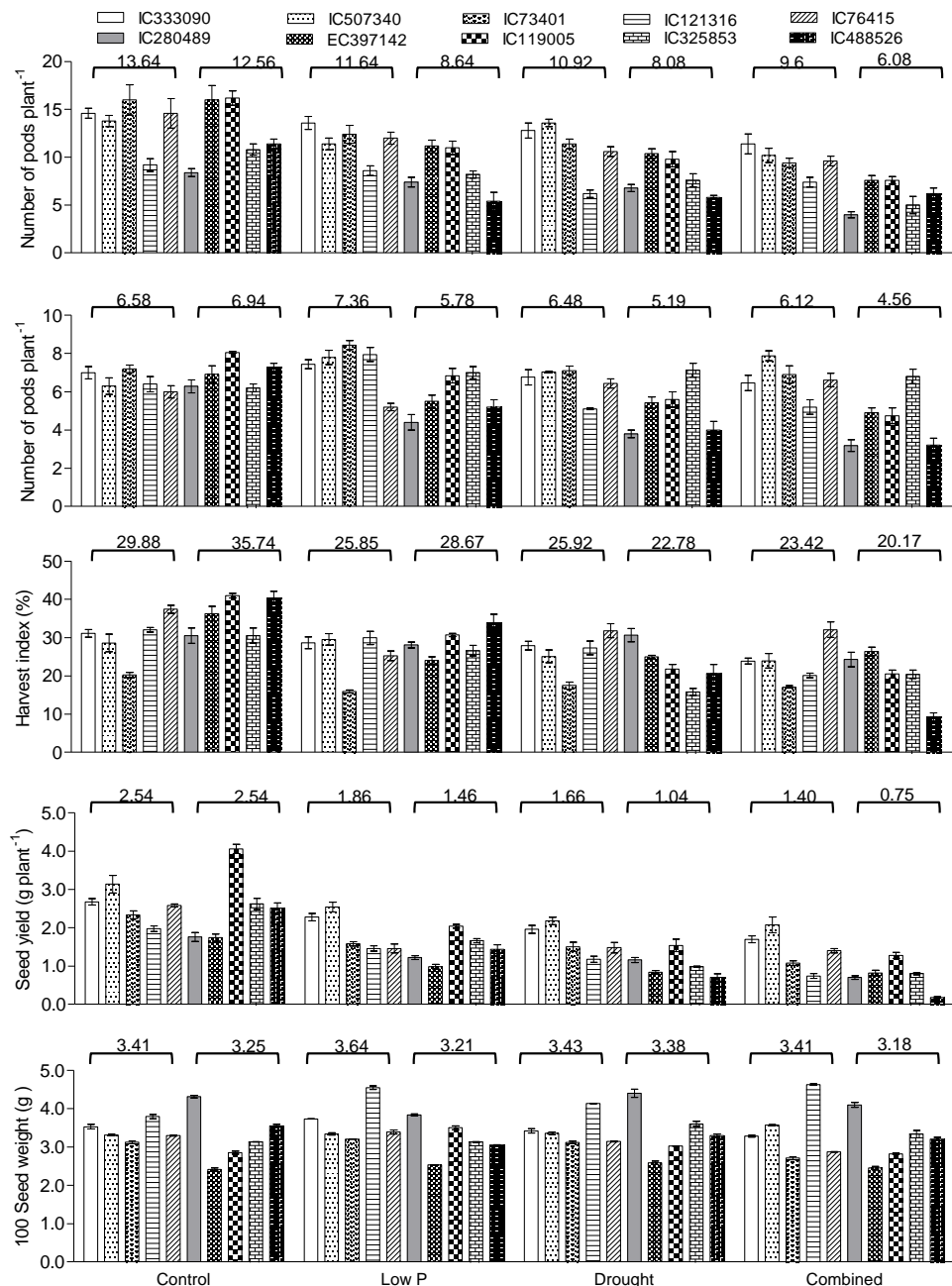
### 3.8 Yield traits influenced by drought and low P stress

All the sources of variability (P, W, and G) had significant ( $P < 0.05$ ) effect on yield and its attributes, except for the number of seed per pod which was found non-significant for P×W interaction (Suppl. Table 4). In general, yield traits showed a significant reduction under all stress treatments in



comparison to control (Fig. 7a-e). Seed yield was the most adversely affected trait whereas 100-seed weight was least affected under low P, drought or combined stress. The number of pods plant<sup>-1</sup>, number of seeds pod<sup>-1</sup>, seed yield and HI were maintained higher in tolerant group as compared to sensitive group under stress treatments.

Among stress treatments, combined stress resulted in drastic reduction in seed yield, maximum reduction being noted in sensitive (70%) rather than the tolerant (45%) group in comparison to control. Among accessions, least reduction was observed in IC507340 (34%) and IC333090 (37%) whereas more than 90% decrease in seed yield was found in IC488526 and IC325853. Similarly, HI decreased by 21 and 44% in tolerant and sensitive groups respectively under combined stress in comparison to control.



**Figure 7.** Effect of low P, drought and combined stress on yield traits in 10 mung bean accessions. Values on bars 1-5 and 6-10 represents mean of tolerant and sensitive accessions respectively. The letters on each bar denotes significant difference between accessions in each treatment. Error bars indicate mean ± SEM.

## 4. Discussion

### 4.1 Interaction of low P and drought on growth and root traits in hydroponics

Trait identification is one of the important steps in selecting the tolerant genotypes for abiotic stresses. Phenotyping of mungbean core set under low P, drought and combined stress was carried out in hydroponics. The PCA revealed the most contributing traits governing genotypic variability and common in all stresses were total plant dry weight, shoot dry weight, root surface area, total root length, moisture retention capacity and plant P uptake. Further, the PCA ranking allowed categorization of accessions into tolerant and sensitive groups. Similar ranking approach was reported in barley, wheat and switchgrass under drought stress [51,54,55].

The shoot and root traits were less affected by low P and drought stress in tolerant group as compared to sensitive group. Our results are in accordance with those reported in lentil phenotyped in hydroponics as well as in soil culture and subjected to drought stress [43]. However, low P significantly reduced the shoot and root dry weight in chickpea [56]. Increased root growth is associated with improved ability of plants to mine soil moisture and nutrients. Our findings are in conformity with studies in lentil [57], green gram [58,59], soybean [60] and wheat [61] indicating low P alters root architectural traits such as total root length, surface area and root-to-shoot ratio. Increased root-to-shoot ratio is associated with higher investment of photosynthates towards root growth which further enhances P acquisition. Low P tolerant pigeon pea (*Cajanus cajan*) genotypes exhibited higher root surface area, total root length, root dry mass, root-to-shoot ratio and leaf P content [62].

The average root diameter in tolerant group was significantly less under low P as compared to sensitive one indicating the importance of more fine roots in P acquisition. Our result is in agreement with reference [63] who also reported lesser root diameter under low P in lentil. It is shown that shallow root system with more basal roots is efficient in P acquisition in soil with less bioavailable P whereas deeper root system contributes to terminal moisture stress condition [64]. Our findings showed that combined stress significantly reduced the shoot and root dry weight, similar to that reported in soybean [65,66].

### 4.2 Combined low P and drought stress has additive effect on organic acid exudation

We observed several folds increase in exudation of total organic acid consistently under low P, drought and combined stress in accessions IC73401, IC121316 and IC333090 as compared to control. Among various organic acids analysed, low P stress induced exudation of maleate,  $\alpha$ -ketoglutarate, oxalate, citrate, succinate, lactate, acetate and fumarate. Root released organic acids have been widely documented as a key physiological strategy to mobilize P in soils containing low bioavailable P [56, 67]. These organic acids could mobilise soil P ranging between 20 to 30 mg P kg<sup>-1</sup> soil whereas root exudates mobilised >40 mg P kg<sup>-1</sup> soil [50]. This difference in soil P mobilization by root exudate and standard organic acids could be due to the fact that exudates are a mixture of many other carboxylates which needs to be explored. Further, genotypic variability in organic acid exudation in response to low P have been reported in chickpea (*Cicer arietinum*) [68], mungbean [58] and soybean [50] among legume crops. A positive correlation between shoot P content and organic acid per plant in chickpea and mungbean were reported [69,70].

Drought stress also induced root exudation of maleate, citrate and lactate in some accessions while acetate and fumarate were exuded in significant amounts in almost all accessions. However, the accessions responded differently to combined stress where succinate was also exuded significantly higher in six out of ten accessions. In IC333090, the additive effect of drought and low P stress was noted for maleate, succinate and fumarate while in IC121316, malonate, lactate and acetate were significantly higher in root exudate. There are very few reports available on drought induced organic acid exudation into rhizosphere [71,72]. In crested wheatgrass (*Agropyron cristatum*) grown in sand, the drought induced exudation was composed of fumaric and succinic acids while oxalic, malic and malonic acids were also detected [71]. On the other hand, corn plants grown hydroponically and osmotic stress imposed by using PEG-6000 resulted in highest exudation of malic acid [72]. They also

detected significant quantities of lactic, acetic, citric, maleic and succinic acids besides a 10-fold increase in fumaric acid in drought sensitive corn genotype. However, these experiments were performed under controlled conditions and the media used was other than the soil. These reports suggest that organic acids exuded under drought or osmotic stress could possibly mobilise the fixed-P, thereby improving the potential for P uptake by plants as the mobility of P in rhizosphere decreases during drought stress. We did not find any report of organic acid exudation in rhizosphere under combined stress of low P and drought grown in soil which may result in altered composition of organic acids in the exudates.

#### 4.3 Combined stress influences water relation, pigment concentration and gas exchange

In soil culture, mungbean accessions showed differential response to RWC and MSI, tolerant ones exhibiting higher values under combined stress. Leaf RWC is a better indicator of plant water status than water potential under abiotic stress [73]. Further, we observed higher MSI values in drought stressed plants supplied with sufficient P than the combined stress. Similar positive effects of P application on African violet (*Saintpaulia ionantha*) plants grown under drought have been attributed to improved water relations and drought tolerance [74]. Increased MSI was found to be associated with osmotic adjustment at higher levels of P nutrition under water stress condition in maize [75].

In the present study, total chlorophyll was significantly reduced under drought stress in comparison to control in sensitive accessions (IC73401 and IC280489). Several reports mentioned a reduction in chlorophyll in several crops exposed to drought stress such as in common bean [20], cluster bean [21], soybean [76], chickpea [77] and mungbean [22]. Although increased chlorophyll concentration is a typical trait under low P stress but we did not find significant change which corroborates with results in common bean [78].

The gas exchange traits ( $A$ ,  $E$  and  $g_s$ ) decreased under drought but the reduction was drastic under combined stress. Stomatal limitations reduced the gaseous exchange between leaf and atmosphere which is the main cause for reduced  $A$ ,  $E$  and  $g_s$ . These corroborates with earlier reports in legume crops such as Fababean [79], chickpea [77], common bean [80,81] and mungbean [22,82] under drought stress. The physiological mechanism has been explained in other studies which states that stomatal closure mediated by ABA during drought stress is the primary limitation of photosynthesis [83,84]. In addition to sensitizing stomata to water stress, low P increased ABA accumulation during moisture stress in cotton [14]. Further, low P availability reduces the  $P_i$  concentration in chloroplast stroma which inhibits ATP synthase activity and cause the lumen acidification due to proton accumulation, ultimately leading to linear electron flow inhibition [85]. Plants with low P also showed a reduction in gas exchange traits in chickpea [86]. Higher P concentration in plants improve instantaneous WUE because it allows a minor suppression of photosynthesis per unit of water transpired [9,87] and maintenance of stomatal conductance [12]. A significant correlation reported between transpiration ratio and leaf P concentration in tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*), and sunflower (*Helianthus annuus*) under sufficient P [88]. Similar to our results, the enhancement in photosynthetic activity through application of phosphatic fertilizer under drought stress was reported in cluster bean [37].

#### 4.4 Influence of combined stress on growth, yield, P uptake and P use efficiency

Accumulation of shoot biomass was adversely affected by low P and drought stress in the present study. Similar reduction in shoot biomass was reported in mungbean [58] and soybean [50] under low P, and less dry matter in mungbean under drought [22]. We also noted reduction in P concentration and P uptake in shoot and grain under low P, drought and combined stress which is obviously due to low tissue P concentration and less soil moisture availability limiting P mobility. Drastic decrease in P uptake under combined stress was the result of less biomass accumulation but differential response of accessions was noted. Shoot biomass and shoot P uptake were the most sensitive traits to P deficiency in common bean and soybean genotypes [89,90]. Similar reduction in P uptake and biomass accumulation in common bean under moisture deficit was reported earlier [91]. Drought stress drastically affects P uptake in wheat plants leading to 62% reduction which is in accordance with our

results [92]. Increased root growth and higher organic acid exudation in accessions IC333090, IC73401 and IC507340 might have resulted in the least reduction in P uptake under drought or combined stress leading to efficient P mobilization.

There are several reports stating significant reduction in yield in mungbean subjected to either drought stress [22,27, 93,94] or low P [58,95]. Our results are in agreement with studies in clover [96], soybean [40,41,65], mungbean [97], and cowpea [98] which showed that P application to legumes exposed to drought improves yield and other attributes. An increased linear relationship was reported at different levels of P fertilization and grain yield and its attributes in chickpea [39] and soybean [41,65]. In cluster bean, faster recovery upon re-watering and enhanced grain yield was noticed in drought stressed plants grown with P as compared to without P fertilization [37].

In our study, the PHI and PUE was significantly affected by low P and drought in mungbean genotypes. PHI decreased under low P and drought conditions however, the combined stress markedly affected the PHI and PUEg in both contrasting groups. The higher PHI and PUEg was found in tolerant accessions rather than sensitive ones (Fig. 5a,b) indicating better translocation and remobilization of photosynthates and P from vegetative to reproductive parts. The PUEbm obtained higher in sensitive group as compared to tolerant where P uptake was less. These results were in conformity with reference [99] who reported a strong negative correlation between PUE for shoot biomass and P uptake in rice. PUEg have been considered as a better trait as compared to PUEbm due its more positive correlation with harvest index as well as PHI [99,100].

## 5. Conclusions

We systematically identified contrasting mungbean accessions from the core set, derived from 1232 diverse germplasms, based on their physiological performance under low P, drought and combined stress conditions. The tolerant accessions, IC333090 and IC507340, identified in this study can be used as 'donors' in the mungbean improvement programs for developing varieties with enhanced agronomic performance under combined stress. Further, the contrasting material may be used to identify the genes/genomic regions by developing mapping populations and design suitable markers to be used in the molecular breeding program. The major contributing traits for combined stress tolerance such as shoot dry weight, RWC, organic acid exudation, grain yield and P uptake can be used as physiological markers for evaluating the germplasm for combined stress tolerance. Further, we confirmed that P application not only increases the PUE, but also improves growth and yield under drought conditions corroborating the role of P in amelioration of adverse effects of water deficit stress.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1),

Figure S1: **(a)** Experimental layout in the Issapur Farm, NBPGR, New Delhi with 1232 diverse mung bean accessions, **(b)** accession showing mid-day wilting, and **(c)** accession tolerant to mid-day wilting.

Figure S2: Growth of mungbean seedlings in hydroponics at different phosphorus concentration to identify the optimum and low P levels. Growth traits such as **(a)** total chlorophyll, **(b)** leaf area, **(c)** total dry weight, and **(d)** root-to-shoot ratio were recorded on 21 days old seedlings in two accessions, MH 421 and IPM 2-3.

Figure S3: Effect of low P, drought and combined stress on concentration of **(a)** total chlorophyll, and **(b)** carotenoid in 10 mungbean accessions. 1-5 and 6-10 represents tolerant and sensitive accessions respectively. Error bars indicate mean  $\pm$  SEM.

Table S1: Passport data of 100 diverse mungbean accessions grown in hydroponics culture to screen for low phosphorus and drought stress tolerance.

Table S2: The significance of sources of variability for accessions (G, 100 accessions), phosphorus (P, 2 levels), moisture (W, 2 levels) and their interaction effects on growth, water balance and root traits of mungbean accessions grown in hydroponics. \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , NS non-significant.

Table S3: The PCA ranking values derived from principal components (PC) 1 to 4 using 14 physiological traits of mungbean under low P, drought and combined stresses.

Table S4: The significance of sources of variability for accessions (G, 10), phosphorus (P, 2 levels), moisture (W, 2 levels) and their interaction effects on growth and yield traits of mungbean accessions grown in soil. \*\*\*P<0.001, \*\*P<0.01, \*P<0.05, NS- not significant

Table S5: The significance of sources of variability for accessions (G, 10), phosphorus (P, 2 levels), moisture (W, 2 levels) and their interaction effects on photosynthetic pigments and gas exchange traits of mungbean accessions grown in soil. \*\*\*P<0.001, \*\*P<0.01, \*P<0.05, NS- not significant

Table S6: The significance of sources of variability for accessions (G, 10), phosphorus (P, 2 levels), moisture (W, 2 levels) and their interaction effect on root organic acid exudation of mungbean accessions grown in hydroponics. \*\*\*P<0.001, \*\*P<0.01, \*P<0.05, NS, not significant

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