



### **Full Length Article**

## **Categorization and Identification of *Brassica* Genotypes for Phosphorus Utilization Efficiency**

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### **Abstract**

Development and introduction of new cultivars with higher phosphorus use efficiency (PUE) is utmost important to uphold crop yields on low-P soils. Categorization of existing germplasm based on growth performance under P-deficiency is necessary for any program aiming to enhance PUE. Ten *Brassica* genotypes (Rainbow, Oscar, R-75/1, R-100/6, R-125/12, Surhan-2012, R-125/17, Con-I, Con-II, and Con-III) were categorized for dry matter (DM) production and PUE in a hydroponic study by growing at two P levels (*i.e.*, deficient, 20  $\mu\text{M}$  Pi and adequate, 200  $\mu\text{M}$  Pi). Genotypes varied significantly for plant growth and biomass related attributes under both P levels. Genotypes Oscar and Con-III produced maximum DM and accumulated more P under deficiency stress and thus proved to be efficient, while genotypes R-75/1 and R-125/17 demonstrated lower values of these indices and were therefore, in-efficient. Four methods were compared using different parameters/indices to categorize genotypes at both P levels. Some genotypes (*i.e.*, Oscar, R-75/1, R-125/17, Con-I) maintained their status regarding P-efficiency when categorized by different methods, while other revealed variable results in response to adequate and/or deficient P level. Categorization of genotypes into four groups based on population means only, had a very narrow range between responsive and non-responsive or efficient and in-efficient genotypes. Therefore, this system may not be more helpful for screening germplasm on a large scale. Conversely, the categorization of genotypes into three classes and their distribution into nine groups was the most excellent approach to distinguish minor differences among genotypes. Moreover, root DM, shoot DM, root-shoot ratio, total P uptake, and PUE were important parameters taken into consideration while categorizing *Brassica* genotypes for P-efficiency. © 2020 Friends Science Publishers

**Keywords:** Biomass yield; *Brassica*; Categorization; Phosphorus efficiency; Stress factor

### **Introduction**

Phosphorus (P) is the second macro-nutrient essential for optimal plant growth and is among the major drivers of sustainable crop productivity worldwide (Stewart *et al.* 2005). Nevertheless, majority of the terrestrial ecosystems have moderate to severe P deficiency, thereby reducing crop yields (Lynch 2011). Higher pH and  $\text{CaCO}_3$  contents are the main causes of low P availability in alkaline calcareous soils, while higher concentrations of iron (Fe) and aluminum (Al) hamper P availability to plants in acidic soils (Wang *et al.* 2015). Addition of commercial P containing fertilizers is generally suggested to maintain optimal P supply to plants (Hussain *et al.* 2016; Shepherd *et al.* 2016; Ijaz *et al.* 2018). However, this approach is not sustainable due to finite and non-renewable phosphate rock reserves, geo-political issues, costly P-fertilizers, and potentially harmful to the environment when used in excess (Cordell *et al.* 2009; Liu 2015), thus compelling the scientists to

improve P-efficiency.

Development of P-efficient cultivars may sustain crop productivity, lower production costs, reduce adverse environmental impacts, and ensure global food security (Aziz *et al.* 2011). The exploitation of P-efficient cultivars cannot be an alternate to chemical fertilizers but could be a complement to P-fertilization depending on native soil conditions (Korkmaz *et al.* 2009). The P-efficient plants producing higher biomass/yield at low P supply are desirable under low-P input systems, while responsive plants which produce more biomass/yield with the addition of P are most valuable under fertilized high-input systems (Gill *et al.* 2004; Yaseen and Malhi 2009). Considerable variations among different field crops have been documented against P-deficiency tolerance *i.e.*, mungbean (*Vigna radiata* R. Wilczek; (Irfan *et al.* 2017), wheat (*Triticum aestivum* L.; Abbas *et al.* 2018; Bilal *et al.* 2018), *Brassica* spp. (Akhtar *et al.* 2008; Aziz *et al.* 2011), rice (*Oryza sativa* L.; Aziz *et al.* 2005, Irfan *et al.* 2019), cotton

(*Gossypium hirsutum* L.; Dorahy et al. 2008), and maize (*Zea mays* L.; Gerhardt et al. 2017). Such variations should be exploited in order to uphold crop production on P impoverished soils. Screening and classification of existing germplasm based on the growth performance under P-deficient environment is a necessary for any breeding venture to produce P-efficient genotypes (Bilal et al. 2018). Screening is also important for the identification of plants suitable for cultivation in soils with varying P contents and also facilitates the selection of parents for recombination breeding to enhance P-efficiency (Ozturk et al. 2003; Gill et al. 2004). In many countries, screening of field crops for P-efficiency have been conducted successfully, but the information from studies in other countries cannot be used in local programs due to variations in locally available germplasm than plant material used in studies conducted outside the country (Chin et al. 2011).

The P-efficiency is either the ability of a genotype to absorb higher P from rooting medium and/or utilization of acquired P to produce more grain yield or biomass. Several methods have been proposed by different scientists to categorize crop germplasm against P-deficiency stress, each having some advantages/disadvantages. According to Gerloff (1977), genotypes can be categorized into four classes based on the efficiency and responsiveness, i.e., efficient and responsive, efficient but non-responsive, inefficient but responsive, and in-efficient and non-responsive. Gill et al. (2004) proposed a classification by creating low, medium, and high groups of efficiency and responsiveness each, with a significant difference between low and high efficient or responsive genotypes. Gerhardt et al. (2017) also classified genotypes for efficiency and responsiveness to P based on the deviation of average shoot DM of each genotype relative to the average shoot DM under each environment i.e., deficient or adequate P. The P-efficiency of crop cultivars differs with the parameters/criteria and methods used for calculating P-efficiency (Ozturk et al. 2005). *Brassica* is an important oilseed crop, particularly sensitive to P-deficiency and need relatively more P than cereals for optimal growth (Akhtar et al. 2008). In current study, four methods were compared using different parameters/indices to categorize *Brassica* genotypes at adequate and deficient P conditions. Moreover, some important parameters were also identified which should be taken into consideration while categorizing *Brassica* genotypes for P-efficiency.

## Materials and Methods

### Hydroponic experiment

Seeds of ten *Brassica* genotypes (Rainbow, Oscar, R-75/1, R-100/6, R-125/12, Surhan-2012, R-125/17, Con-I, Con-II, and Con-III) were kindly provided by Plant Breeding and Genetics Division of Nuclear Institute of Agriculture (NIA) Tandojam – Pakistan. Seeds were surface sterilized, washed

with distilled water and then sown in polythene lined metal trays having two inches layer of pre-washed sand. After seven days of seed germination, uniform seedlings of all genotypes were transferred to nutrient solution. The root systems of the seedlings were carefully rinsed with distilled water prior to transferring in order to remove adhering sand. The full-strength nutrient solution (pH 5.5) contained 5.0 mM nitrogen, 3.5 mM potassium, 1.5 mM calcium, 0.5 mM magnesium, 2.15 mM sulfur, 50  $\mu$ M chlorides, 0.5  $\mu$ M molybdenum, 25.0  $\mu$ M boron, 2.0  $\mu$ M zinc, 2.0  $\mu$ M manganese, 0.5  $\mu$ M copper, and 50.0  $\mu$ M iron. The seedlings were supported by foam plugs in the holes of thermopole sheet fixed at the top of plastic containers following two factor completely randomized design with three replications. Plants were allowed to grow at two P levels i.e., adequate (200  $\mu$ M Pi) and deficient (20  $\mu$ M Pi), maintained using potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ). Nutrient solution was replaced with fresh solution after every week in order to ensure continuous supply of nutrients, and pH was adjusted daily at  $5.5 \pm 0.2$  units. Plants were harvested from both P groups after 28 days of transplanting. Samples (roots, shoots) were oven dried at 70°C for 48 hours, and stored under desiccation until recording of dry weights.

Oven dried samples were grinded using Wiley's mill to pass through a 0.42 mm screen. Samples were wet digested using di-acid mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$  (5:1, v/v). The total P concentration was estimated by spectrophotometer at 470 nm wavelength (Chapman and Pratt 1961). Following P relations were calculated using formulae:

$$\begin{aligned} \text{P uptake (mg plant}^{-1}\text{)} &= \text{P concentration (mg g}^{-1}\text{)} \\ &\times \text{RDM or SDM (g plant}^{-1}\text{)} \\ \text{P – utilization efficiency (mg}^2\text{ SDM } \mu\text{g P}^{-1}\text{)} &= \frac{\text{SDM (g plant}^{-1}\text{)}}{\text{P concentration (mg g}^{-1}\text{)}} \times 1000 \\ \text{P – stress factor (\%)} &= \frac{\text{SDM}_{\text{adequate P}} - \text{SDM}_{\text{deficient P}}}{\text{SDM}_{\text{adequate P}}} \\ &\times 100 \end{aligned}$$

Where 'RDM' is the root dry matter and 'SDM' is the shoot dry matter.

### Categorization methods

**Method 1:** According to this method, categorization of *Brassica* genotypes was performed using DM production and PUE at deficient and adequate P levels (Fageria 1993). Genotypes producing DM higher than the average DM were efficient, while those having PUE above the average PUE were declared responsive and vice versa. In this way, genotypes were divided into four categories based on efficiency and response i.e., ER: efficient and responsive, IR:

in-efficient but responsive, ENR: efficient but non-responsive, and INR: in-efficient and non-responsive.

**Method 2:** Genotypes were categorized according to Gerhardt *et al.* (2017) for efficiency and responsiveness to P based on the deviation of average SDM of each genotype relative to the average SDM under each environment *i.e.*, deficient or adequate P. The obtained values were plotted in a scatter graph, representing deviation under high P level (responsiveness) on *x*-axis and deviation under low P level (efficiency) on *y*-axis. The expression used to categorize genotypes for P-efficiency was  $E_p = M_{GD} - M_{ED}$ , where  $M_{GD}$  represents the average SDM of individual genotype with deficient P and  $M_{ED}$  represents average SDM of environment with deficient P level. Likewise, the equation adopted to categorize genotypes for P-responsiveness was  $R_p = M_{GA} - M_{EA}$ , where  $M_{GA}$  represents the average SDM of individual genotype with adequate P and  $M_{EA}$  represents average SDM of the environment with adequate P level. This method also creates four categories *i.e.*, ER, IR, ENR, and INR.

**Method 3:** This method distributed genotypes into nine groups by constructing a graph where SDM was represented on the *x*-ordinate, while shoot P uptake on the *y*-ordinate (Gill *et al.* 2004). Each ordinate was dissected into three groups *i.e.*, low (if mean is  $<\mu - SD$ ), medium (if mean is between  $\mu - SD$  to  $\mu + SD$ ), and high (if mean is  $>\mu + SD$ ). Where ' $\mu$ ' is the population mean and 'SD' is the standard deviation.

**Method 4:** According to Gill *et al.* (2004), *Brassica* genotypes were categorized into efficient, medium, and in-efficient genotypes based on the index scores for different indices (*i.e.* root DM, shoot DM, root-shoot ratio, total P uptake, and PUE) at both P supplies. This categorization is based on the absolute values of each genotype with the population mean ( $\mu$ ) and standard deviation (SD) for each parameter (Gill *et al.* 2004). The genotypes are assigned numbers (1, 2, and 3) as 1 for in-efficient (if mean is  $<\mu - SD$ ), 2 for medium (if mean is between  $\mu - SD$  to  $\mu + SD$ ), and 3 for efficient (if mean is  $>\mu + SD$ ). Where ' $\mu$ ' is the population mean and 'SD' is the standard deviation.

### Statistical analysis

The obtained data was analyzed statistically by software Statistix 8.1 according to methods of Steel *et al.* (1997). A completely randomized design with factorial arrangements was used for the analysis of variance and least significant difference test at 5% probability level was employed to separate the significant differences among treatment means. Graphical presentation of data to categorize *Brassica* genotypes was carried out using Microsoft Excel.

## Results

### Plant Growth and Dry Matter Production

All the plant growth and biomass related parameters of

*Brassica* genotypes were significantly affected under adequate and deficient P supply (Table 1). Plant height of different genotypes varied from 15.0 cm (R-75/1) to 19.5 cm (Surhan-2012) under deficient P conditions and enhanced significantly under adequate P supply, which ranged from 35.3 cm (R-125/17) to 44.0 cm (R-100/6). In all genotypes, the mean root length increased in response to P-deficiency than at adequate P level. Root length ranged from 36.5 cm (R-75/1) to 43.8 cm (Con-III) under deficient P level. Averaged across all genotypes, root DM increased from 0.22 g plant<sup>-1</sup> at P deficient level to 0.25 g plant<sup>-1</sup> under P adequate supply. *Brassica* genotypes R-100/6 and Con-I produced higher root DM (0.27 and 0.30 g plant<sup>-1</sup>) in response to deficient and sufficient P conditions, respectively. Mean shoot DM declined from 2.18 g plant<sup>-1</sup> at adequate P to 0.64 g plant<sup>-1</sup> under P deficiency. Con-III produced maximum shoot DM (0.85 g plant<sup>-1</sup>) at deficient P, however genotype Oscar showed higher shoot DM (2.92 g plant<sup>-1</sup>) at adequate P level. Genotypes also showed variable response for total DM (root + shoot), with maximum value (3.16 g plant<sup>-1</sup>) by Oscar under sufficient P level, while 1.10 g plant<sup>-1</sup> by Con-III at deficient P. The P-stress factor (PSF) which indicates the percent reduction in DM production in response to P deficiency is depicted in Fig. 1. The tested genotypes varied considerably for PSF with mean value of 68.48%. The highest value of PSF was recorded in genotype R-125/12 (76.59%), while lowest was observed in R-75/1 (61.49%). Genotypes also differed considerably for root-shoot ratio (RSR) at both P levels (Table 2). The RSR varied from 0.22 (Oscar) to 0.54 (Con-I), with mean value of 0.36 under P deficiency and 0.09 (Oscar) to 0.17 (Con-I), with average of 0.13 at adequate P supply.

### Phosphorus accumulation

The data pertaining to root, shoot, and total P uptake is presented in Table 2. Phosphorus levels and genotypes had significant main and interactive effects regarding above mentioned parameters. Genotypes showed substantial variations for root P uptake (RPU) at both P supplies. The RPU varied from 0.34 to 0.88 mg plant<sup>-1</sup> at deficient P and 1.10 – 2.05 mg plant<sup>-1</sup> at adequate P supply. Averaged of all genotypes, RPU at adequate P was almost three folds higher than at deficient P level. The genotype Con-I accumulated the highest RPU (0.88 mg plant<sup>-1</sup>), while genotype R-75/1 revealed the lowest RPU (0.34 mg plant<sup>-1</sup>) in response to P deficiency. The shoot P uptake (SPU) ranged between 9.12 mg plant<sup>-1</sup> (R-75/1) to 16.0 mg plant<sup>-1</sup> (Oscar), with mean value of 12.35 mg plant<sup>-1</sup> at adequate P level and 0.60 mg plant<sup>-1</sup> (R-75/1) to 1.58 mg plant<sup>-1</sup> (Con-III), with mean of 0.99 mg plant<sup>-1</sup> at P deficiency. *Brassica* genotypes also varied substantially for total PU (RPU + SPU) at each P supply. Averaged over genotypes, TPU was about > 8 fold lower under P deficiency as compared with adequate P supply. The genotype Con-III showed the highest TPU (2.30 mg plant<sup>-1</sup>), while genotype R-75/1 accumulated the

**Table 1:** Interactive effect of genotypes and phosphorus levels on plant height, root length, and dry matter [root, shoot, total (root + shoot)] of *Brassica*

Genotypes	Plant height (cm)		Root length (cm)		Root dry matter (g/plant)		Shoot dry matter (g/plant)		Total dry matter (g/plant)	
	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>
Rainbow	16.3 ± 0.73 <sup>f</sup>	36.0 ± 1.32 <sup>d</sup>	41.3 ± 2.03 <sup>ab</sup>	37.8 ± 1.30 <sup>bc</sup>	0.26 ± 0.04 <sup>ad</sup>	0.29 ± 0.02 <sup>a</sup>	0.69 ± 0.02 <sup>ef</sup>	2.25 ± 0.02 <sup>bd</sup>	0.94 ± 0.01 <sup>ef</sup>	2.54 ± 0.04 <sup>bc</sup>
Oscar	15.7 ± 1.20 <sup>df</sup>	36.7 ± 0.67 <sup>cd</sup>	36.7 ± 2.41 <sup>be</sup>	31.7 ± 0.33 <sup>ef</sup>	0.17 ± 0.01 <sup>de</sup>	0.24 ± 0.02 <sup>ad</sup>	0.77 ± 0.06 <sup>ef</sup>	2.92 ± 0.49 <sup>a</sup>	0.93 ± 0.06 <sup>ef</sup>	3.16 ± 0.47 <sup>a</sup>
R-75/1	15.0 ± 0.87 <sup>de</sup>	41.3 ± 1.17 <sup>ab</sup>	36.5 ± 2.57 <sup>be</sup>	27.0 ± 1.73 <sup>f</sup>	0.11 ± 0.01 <sup>e</sup>	0.17 ± 0.01 <sup>c-c</sup>	0.42 ± 0.05 <sup>f</sup>	1.50 ± 0.48 <sup>e</sup>	0.53 ± 0.05 <sup>g</sup>	1.68 ± 0.47 <sup>ef</sup>
R-100/6	15.5 ± 0.76 <sup>df</sup>	44.0 ± 1.16 <sup>a</sup>	41.0 ± 1.73 <sup>ab</sup>	39.0 ± 2.08 <sup>ac</sup>	0.27 ± 0.05 <sup>ad</sup>	0.30 ± 0.03 <sup>a</sup>	0.62 ± 0.07 <sup>ef</sup>	2.28 ± 0.46 <sup>bd</sup>	0.88 ± 0.03 <sup>ef</sup>	2.58 ± 0.43 <sup>ac</sup>
R-125/12	16.2 ± 1.20 <sup>f</sup>	42.2 ± 1.59 <sup>ab</sup>	41.5 ± 1.26 <sup>ab</sup>	37.7 ± 2.91 <sup>bd</sup>	0.20 ± 0.02 <sup>ac</sup>	0.21 ± 0.02 <sup>ac</sup>	0.51 ± 0.04 <sup>f</sup>	2.20 ± 0.07 <sup>bd</sup>	0.72 ± 0.06 <sup>g</sup>	2.41 ± 0.09 <sup>bd</sup>
Surhan-2012	19.5 ± 1.32 <sup>e</sup>	35.7 ± 0.33 <sup>d</sup>	37.8 ± 1.97 <sup>bc</sup>	32.3 ± 1.86 <sup>df</sup>	0.21 ± 0.03 <sup>ac</sup>	0.28 ± 0.01 <sup>ab</sup>	0.63 ± 0.03 <sup>ef</sup>	2.16 ± 0.08 <sup>bd</sup>	0.84 ± 0.04 <sup>ef</sup>	2.44 ± 0.08 <sup>bd</sup>
R-125/17	15.5 ± 1.53 <sup>df</sup>	35.3 ± 0.88 <sup>d</sup>	37.5 ± 0.50 <sup>bd</sup>	36.3 ± 1.86 <sup>be</sup>	0.19 ± 0.01 <sup>be</sup>	0.18 ± 0.08 <sup>c-c</sup>	0.52 ± 0.02 <sup>f</sup>	1.73 ± 0.36 <sup>de</sup>	0.71 ± 0.01 <sup>e-g</sup>	1.90 ± 0.31 <sup>de</sup>
Con-I	17.2 ± 0.44 <sup>ef</sup>	39.3 ± 1.92 <sup>bc</sup>	37.3 ± 1.77 <sup>bd</sup>	34.7 ± 1.20 <sup>ce</sup>	0.27 ± 0.08 <sup>ac</sup>	0.30 ± 0.01 <sup>a</sup>	0.60 ± 0.12 <sup>f</sup>	1.82 ± 0.17 <sup>c-e</sup>	0.87 ± 0.07 <sup>ef</sup>	2.12 ± 0.17 <sup>c-e</sup>
Con-II	16.0 ± 0.29 <sup>f</sup>	39.8 ± 0.44 <sup>bc</sup>	41.0 ± 1.73 <sup>ab</sup>	38.3 ± 1.67 <sup>bc</sup>	0.25 ± 0.05 <sup>ad</sup>	0.28 ± 0.01 <sup>ab</sup>	0.80 ± 0.01 <sup>de</sup>	2.39 ± 0.19 <sup>ac</sup>	1.06 ± 0.05 <sup>e</sup>	2.68 ± 0.18 <sup>ac</sup>
Con-III	16.3 ± 0.93 <sup>f</sup>	41.2 ± 1.92 <sup>ab</sup>	43.8 ± 2.62 <sup>a</sup>	38.5 ± 2.37 <sup>ac</sup>	0.25 ± 0.04 <sup>ad</sup>	0.26 ± 0.02 <sup>ad</sup>	0.85 ± 0.09 <sup>de</sup>	2.50 ± 0.20 <sup>ab</sup>	1.10 ± 0.10 <sup>e</sup>	2.76 ± 0.18 <sup>ab</sup>
LSD value at 5%	3.25	5.44	0.10	0.10	0.63	0.59				

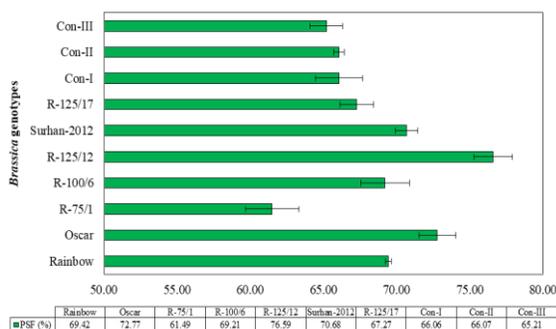
Values are means of three replications ± standard error (SE)

Genotypic means not sharing similar letter(s) under each parameter differ significantly from each other (LSD test, P ≤ 0.05)

**Table 2:** Interactive effect of genotypes and phosphorus levels on root-shoot ratio, P uptake [root, shoot P uptake, total (root + shoot)], and P-utilization efficiency of *Brassica*

Genotypes	Root-shoot ratio		Root P uptake (mg/plant)		Shoot P uptake (mg/plant)		Total P uptake (mg/plant)		P-utilization efficiency (mg <sup>2</sup> SDM μg P <sup>-1</sup> )	
	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>	P <sub>deficient</sub>	P <sub>adequate</sub>
Rainbow	0.38 ± 0.07 <sup>ac</sup>	0.13 ± 0.01 <sup>e-g</sup>	0.71 ± 0.08 <sup>e-g</sup>	1.89 ± 0.09 <sup>ab</sup>	0.88 ± 0.03 <sup>ef</sup>	11.60 ± 0.13 <sup>bd</sup>	1.59 ± 0.11 <sup>ef</sup>	13.49 ± 0.18 <sup>bd</sup>	539.7 ± 51.1 <sup>a</sup>	435.1 ± 14.5 <sup>ac</sup>
Oscar	0.22 ± 0.01 <sup>c-g</sup>	0.09 ± 0.02 <sup>g</sup>	0.53 ± 0.02 <sup>fg</sup>	1.76 ± 0.11 <sup>ac</sup>	1.10 ± 0.12 <sup>de</sup>	16.00 ± 2.74 <sup>a</sup>	1.62 ± 0.13 <sup>ef</sup>	17.76 ± 2.63 <sup>a</sup>	545.3 ± 61.9 <sup>a</sup>	532.8 ± 87.3 <sup>a</sup>
R-75/1	0.28 ± 0.04 <sup>bc-g</sup>	0.15 ± 0.06 <sup>d-g</sup>	0.34 ± 0.01 <sup>g</sup>	1.47 ± 0.11 <sup>bd</sup>	0.60 ± 0.09 <sup>f</sup>	9.12 ± 2.89 <sup>d</sup>	0.94 ± 0.09 <sup>g</sup>	10.59 ± 2.78 <sup>d</sup>	295.4 ± 35.2 <sup>df</sup>	247.3 ± 79.1 <sup>f</sup>
R-100/6	0.46 ± 0.12 <sup>ab</sup>	0.15 ± 0.04 <sup>d-g</sup>	0.64 ± 0.12 <sup>ef</sup>	2.05 ± 0.22 <sup>a</sup>	0.85 ± 0.12 <sup>ef</sup>	12.04 ± 2.43 <sup>bd</sup>	1.49 ± 0.12 <sup>ef</sup>	14.08 ± 2.22 <sup>bc</sup>	451.9 ± 48.9 <sup>ac</sup>	431.8 ± 86.5 <sup>ad</sup>
R-125/12	0.39 ± 0.02 <sup>bc</sup>	0.09 ± 0.01 <sup>g</sup>	0.59 ± 0.08 <sup>g</sup>	1.40 ± 0.09 <sup>cd</sup>	0.72 ± 0.09 <sup>f</sup>	14.04 ± 0.44 <sup>ab</sup>	1.31 ± 0.16 <sup>e-g</sup>	15.43 ± 0.51 <sup>ab</sup>	365.5 ± 20.1 <sup>bf</sup>	345.9 ± 11.5 <sup>bf</sup>
Surhan-2012	0.33 ± 0.05 <sup>bd</sup>	0.13 ± 0.01 <sup>e-g</sup>	0.53 ± 0.05 <sup>fg</sup>	1.63 ± 0.06 <sup>ac</sup>	0.79 ± 0.03 <sup>f</sup>	10.42 ± 0.39 <sup>cd</sup>	1.33 ± 0.06 <sup>e-g</sup>	12.06 ± 0.35 <sup>cd</sup>	505.3 ± 34.2 <sup>a</sup>	446.9 ± 16.4 <sup>ac</sup>
R-125/17	0.37 ± 0.03 <sup>bc</sup>	0.13 ± 0.08 <sup>e-g</sup>	0.49 ± 0.05 <sup>fg</sup>	1.10 ± 0.17 <sup>de</sup>	0.78 ± 0.09 <sup>f</sup>	9.35 ± 1.98 <sup>d</sup>	1.27 ± 0.07 <sup>e-g</sup>	10.45 ± 1.65 <sup>d</sup>	344.9 ± 16.9 <sup>bf</sup>	318.7 ± 66.8 <sup>c-f</sup>
Con-I	0.54 ± 0.12 <sup>a</sup>	0.17 ± 0.01 <sup>d-g</sup>	0.88 ± 0.18 <sup>e</sup>	1.60 ± 0.07 <sup>ac</sup>	1.23 ± 0.24 <sup>de</sup>	12.55 ± 1.20 <sup>ad</sup>	2.11 ± 0.18 <sup>de</sup>	14.14 ± 1.19 <sup>bc</sup>	289.9 ± 55.0 <sup>ef</sup>	264.0 ± 22.9 <sup>f</sup>
Con-II	0.32 ± 0.06 <sup>bc</sup>	0.12 ± 0.01 <sup>fg</sup>	0.82 ± 0.19 <sup>ef</sup>	1.84 ± 0.10 <sup>ac</sup>	1.42 ± 0.10 <sup>d</sup>	13.72 ± 1.04 <sup>ac</sup>	2.24 ± 0.24 <sup>d</sup>	15.57 ± 0.94 <sup>ab</sup>	456.7 ± 20.2 <sup>ac</sup>	417.5 ± 34.1 <sup>ae</sup>
Con-III	0.29 ± 0.05 <sup>bc-f</sup>	0.11 ± 0.01 <sup>fg</sup>	0.72 ± 0.13 <sup>e-g</sup>	1.91 ± 0.14 <sup>ab</sup>	1.58 ± 0.16 <sup>d</sup>	14.62 ± 1.25 <sup>ab</sup>	2.30 ± 0.17 <sup>d</sup>	16.53 ± 1.11 <sup>ab</sup>	463.7 ± 51.1 <sup>ab</sup>	427.6 ± 31.2 <sup>ae</sup>
LSD value at 5%	0.19	0.46	3.51	3.28	138.22					

Values are means of three replications ± standard. Error Genotypic means not sharing similar letter(s) under each parameter differ significantly from each other (LSD test, P ≤ 0.05)



**Fig. 1:** Percent reduction in dry matter production in response to P deficiency (P-stress factor) of various *Brassica* genotypes. Values are means of three replications and are presented with standard errors

lowest TPU (0.94 mg plant<sup>-1</sup>) under P deficiency. At adequate P, genotypes Oscar (17.76 mg plant<sup>-1</sup>) and Con-III (16.53 mg plant<sup>-1</sup>) showed better performance for TPU than other genotypes.

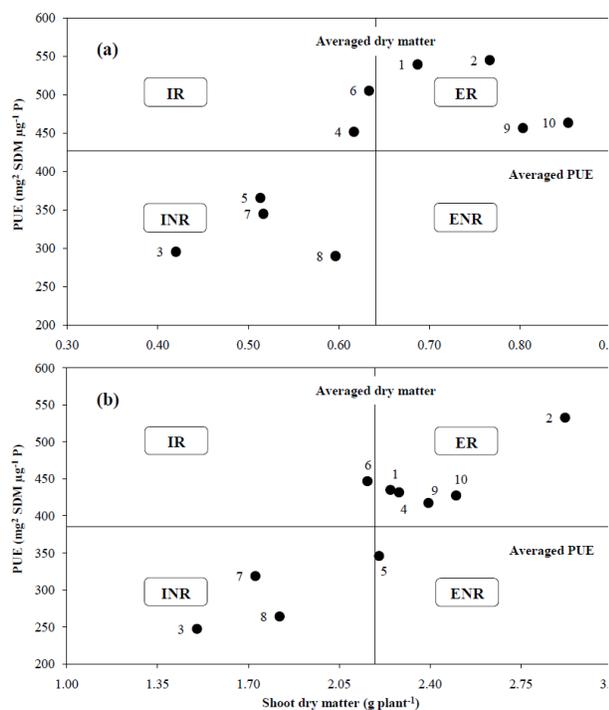
**Phosphorus utilization efficiency (PUE)**

Phosphorus levels and *Brassica* genotypes had significant

main and interactive effects for PUE under solution culture (Table 2). Numerically, the PUE increased substantially in response to P deficiency as compared with adequate P supply. Overall, the magnitude of difference varied from 386.8 – 425.8 mg<sup>2</sup> SDM μg P<sup>-1</sup> when P supply changed from deficient to adequate. Under P deficiency, the genotype Oscar showed the highest PUE (545.3 mg<sup>2</sup> SDM μg P<sup>-1</sup>) followed by Rainbow (539.7 mg<sup>2</sup> SDM μg P<sup>-1</sup>) and Surhan-2012 (505.3 mg<sup>2</sup> SDM μg P<sup>-1</sup>), while genotype Con-I showed the lowest PUE (289.9 mg<sup>2</sup> SDM μg P<sup>-1</sup>). The genotype Oscar also showed greater PUE (532.8 mg<sup>2</sup> SDM μg P<sup>-1</sup>) at adequate P, while genotype R-75/1 showed minimum PUE (247.3 mg<sup>2</sup> SDM μg P<sup>-1</sup>).

**Categorization of *Brassica* genotypes**

**Method 1:** Genotypes were categorized into four groups based on PUE and DM production at both P supplies, as shown in Fig. 2. Genotypes showing PUE over the mean PUE were declared responsive, while those producing DM higher than the average DM were called efficient and vice versa. Genotypes Rainbow, Oscar, Con-II, and Con-III were categorized as ER, genotypes R-75/1, R-125/17, Con-I as INR, while genotype Surhan-2012 was placed in IR category at both P supplies. Nevertheless, R-100/6 was

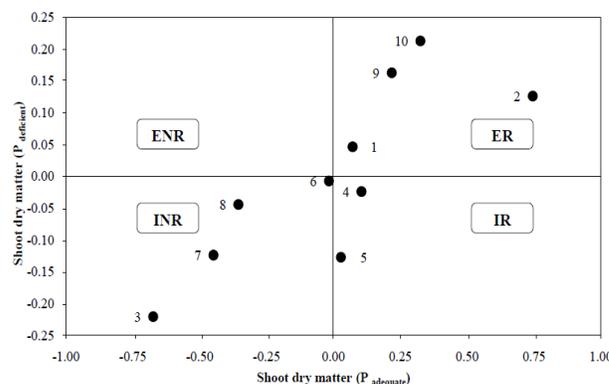


**Fig. 2:** Categorization of *Brassica* genotypes based on P utilization efficiency and shoot dry matter under (a) deficient P, and (b) adequate P supply according to Fageria (1993). This categorization divides genotypes into four categories *i.e.*, ER = efficient and responsive; IR = in-efficient but responsive; ENR = efficient but non-responsive; INR = in-efficient and non-responsive. Numbers in Fig. represent names of *Brassica* genotypes studied in the experiment as 1 = Rainbow; 2 = Oscar; 3 = R-75/1; 4 = R-100/6; 5 = R-125/12; 6 = Surhan-2012; 7 = R-125/17; 8 = Con-I; 9 = Con-II; 10 = Con-III

ranked as ER under P deficiency but shifted to IR category at adequate P level. Likewise, genotype R-125/12 altered its category from INR to ENR when P status changed from deficient to adequate level.

**Method 2:** *Brassica* genotypes were categorized for P-efficiency and responsiveness based on the deviation of average shoot DM of each genotype relative to the average shoot DM under each environment *i.e.*, deficient or adequate P level (Fig. 3). The graph revealed that genotypes Rainbow, Oscar, Con-II, and Con-III were distributed in the quadrant categorized as ER exhibiting shoot DM greater than the mean found in the environments of adequate and deficient P supply. The genotypes R-75/1, Surhan-2012, R-125/17, and Con-I were classified as INR displaying their lower shoot DM than mean under both B environments. The genotypes R-100/6 and R-125/12 were placed in the quadrant classified as IR.

**Method 3:** This method showed variable results in response to with or without P addition (Fig. 4). The genotypes Con-II and Con-III rated as HDM-HP under P deficiency shifted to MDM-MP group at adequate P nutrition. The genotype R-



**Fig. 3:** Categorization of *Brassica* genotypes according to Gerhardt *et al.* (2017) for efficiency and responsiveness to phosphorus based on the deviation of average shoot dry matter of each genotype relative to the average shoot dry matter under each environment *i.e.*, deficient or adequate P. This categorization also divides genotypes into four categories *i.e.*, ER = efficient and responsive; IR = in-efficient but responsive; ENR = efficient but non-responsive; INR = in-efficient and non-responsive. Numbers in Fig. represent names of *Brassica* genotypes studied in the experiment as 1 = Rainbow; 2 = Oscar; 3 = R-75/1; 4 = R-100/6; 5 = R-125/12; 6 = Surhan-2012; 7 = R-125/17; 8 = Con-I; 9 = Con-II; 10 = Con-III

125/17 from MDM-MP group at deficient P altered its position to LDM-LP in relation to P addition. Similarly, genotype Oscar initially placed in MDM-MP category under P deficiency shifted to HDM-HP at adequate P level. However, genotypes Rainbow, R-100/6, R-125/12, Surhan-2012, and Con-I were grouped as MDM-MP under both P environments.

**Method 4:** The *Brassica* genotypes were ranked by allotting index scores as 1 for in-efficient, 2 for medium, and 3 for efficient for various parameters at both P levels (Table 3). The genotypes behaved differentially for each attribute under similar P environment. The genotypes Con-I, Con-II, and Con-III gained maximum index score (12 out of 15), whereas R-75/1 gained least total index score (6 out of 15) under P deficiency. At adequate P, genotype Oscar obtained maximum score (12 out of 15), whereas genotype R-75/1 obtained minimum score (6 out of 15). For each genotype, total index scores at both P supplies were further added up to evaluate the overall performance of tested *Brassica* genotypes. The genotype Con-III obtained highest total score (23 out of 30) followed by genotypes Oscar, R-100/6, Con-I, and Con-II (22 out of 30), while genotype R-75/1 had lowest total score (12 out of 30).

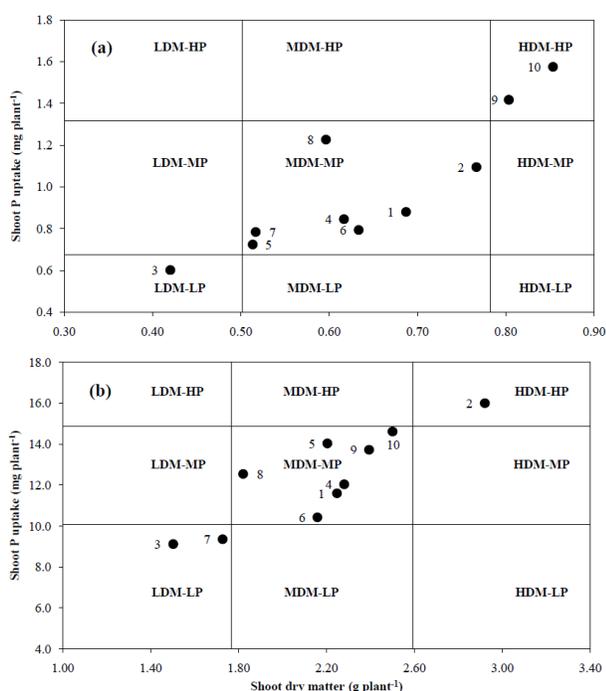
## Discussion

Inter and intra-specific variation exists among field crops for growth performance and P use under adequate and deficient P conditions (Aziz *et al.* 2011; Abbas *et al.* 2018). The understanding of genetic variability regarding P-efficiency

**Table 3:** Categorization of *Brassica* genotypes into efficient (E), medium (M), and in-efficient (I) scoring genotypes based on their index scores of various parameters at deficient and adequate P levels

Genotypes	Root dry matter		Shoot dry matter		Root-shoot ratio		Total P uptake		P-utilization efficiency		Total Score		
	P deficient	P adequate	P deficient	P adequate	P deficient	P adequate	P deficient	P adequate	P deficient	P adequate	Score at deficient P level/ out of 15	Score at adequate P level/ out of 15	Total score/ out of 30
Rainbow	M	M	M	M	M	M	M	M	E	M	11	10	21
Oscar	M	M	M	E	I	I	M	E	E	E	10	12	22
R-75/1	I	I	I	I	M	M	I	I	I	I	6	6	12
R-100/6	M	E	M	M	E	M	M	M	M	M	11	11	22
R-125/12	M	M	M	M	M	I	M	M	M	M	10	9	19
Surhan-2012	M	M	M	M	M	M	M	M	M	M	10	10	20
R-125/17	M	I	M	I	M	M	M	I	M	M	10	7	17
Con-I	E	M	M	M	E	E	E	M	I	I	12	10	22
Con-II	M	M	E	M	M	M	E	M	M	M	12	10	22
Con-III	M	M	E	M	M	M	E	E	M	M	12	11	23

This categorization is based on the absolute values of each genotype with the population mean ( $\mu$ ) and standard deviation (SD) for each parameter according to Gill et al. (2004). The genotypes are scored as in-efficient if their mean is  $< \mu - SD$ , medium if mean is  $\mu - SD$  to  $\mu + SD$ , and efficient if the mean is  $> \mu + SD$



**Fig. 4:** Categorization of *Brassica* genotypes based on shoot dry matter and P uptake and under (a) deficient P, and (b) adequate P supply according to Gill et al. (2004). This categorization divides genotypes into nine categories i.e., **LDM-LP** = low dry matter-low P; **LDM-MP** = low dry matter-medium P; **LDM-HP** = low dry matter-high P; **MDM-LP** = medium dry matter-low P; **MDM-MP** = medium dry matter-medium P; **MDM-HP** = medium dry matter-high P; **HDM-LP** = high dry matter-low P; **HDM-MP** = high dry matter-medium P; **HDM-HP** = high dry matter-high P. Numbers in Fig. represent names of *Brassica* genotypes studied in the experiment as **1** = Rainbow; **2** = Oscar; **3** = R-75/1; **4** = R-100/6; **5** = R-125/12; **6** = Surhan-2012; **7** = R-125/17; **8** = Con-I; **9** = Con-II; **10** = Con-III

and responsiveness is a fundamental step for inheritance studies and for the implementation of a suitable breeding

program to develop P-efficient cultivars (Cock et al. 2002). In present study, the significant interactions among P levels and genotypes for plant height, root length, shoot DM, and P uptake suggested that genotypes may performed differentially under changing P environments (Table 1). Thus it is important to evaluate genotypes under various P supplies if these attributes are being considered as selection criteria. Several genotypes showed better performance under P deficiency due to improved P-use efficiency (PUE). The PUE explains the ability of a genotype to produce biomass and/or grain yield per unit of absorbed P (Yaseen and Malhi 2009). The genotype R-75/1 with minimum value of PUE produced lower DM and accumulated less P under stress conditions and therefore was in-efficient. Thus the productivity of such genotypes could only be sustained if grown on soils with adequate P contents. High yielding genotypes i.e., Oscar and Con-III showed higher PUE under P deficiency and relatively lower values under adequate P supply were efficient in terms of P acquisition to produce biomass under P stress. Efficient genotypes producing higher DM under both P environments are desirable for cultivation on soil with diverse P contents (Irfan et al. 2017; Bilal et al. 2018).

In current study, efficient genotypes i.e., Oscar and Con-III had higher P uptake under P stress than in-efficient genotypes i.e., R-75/1. Moreover, a linear relationship between P uptake and DM also justified this argument. Wide variation found for P uptake and its utilization elucidates the genetic variability and complexity of P-efficiency mechanisms (Hayes et al. 2004). Higher DM by efficient genotypes under P stress was mainly attributed to the establishment of a greater root length, root-shoot ratio (RSR) and total P uptake, thus favoring P acquisition by plants. The genotype Con-III had deepest roots and produced more root DM when exposed to P deficiency stress. According to Ma et al. (2001), root length and root hair density enhanced significantly under P stress for better P absorption. Bates and Lynch (2001) also reported that increased root growth is associated with better plant growth

at low P by exploring larger soil volume. The RSR is an indication of the biomass distribution into roots and shoots. As plant growth is suppressed under low P but the relative effect is most dominant in the case of shoot growth thereby increasing RSR (Lu *et al.* 1999). By comparing different parameters of genotypes, it is evident that efficient genotypes showed better performance in terms of DM production and total P uptake than in-efficient genotypes (R-75/1 and R-125/17). Such variations have great significance for cultivar development as these traits are heritable and can be exploited in breeding programs to enhance P-efficiency (Yaseen and Malhi 2009; Aziz *et al.* 2011).

According to categorization by Fageria (1993), *Brassica* genotypes were distributed into four classes based on efficiency and responsiveness to P *i.e.*, ER, IR, ENR, and INR (Fig. 2). The genotypes in ER category (*i.e.*, Rainbow, Oscar, Con-II, and Con-III) are most desirable as they can produce higher biomass at both P supplies. The genotype R-100/6 was rated as ER under P deficiency but in IR category at adequate P level. Likewise, genotype R-125/12 shifted from INR to ENR when P level altered from deficient to adequate, suggesting that categorization at adequate P is also essential instead at deficient P alone. This method facilitates to recognize those genotypes suitable for cultivation on wide range of P supply (Isherwood 2003). However, this method is based on population means only, so it has a very narrow range between responsive and non-responsive or efficient and in-efficient group. For example, genotypes R-100/6 and Surhan-2012 (at deficient P) and genotypes R-125/12 and Surhan-2012 (at adequate P) were positioned close the boundary line of efficient and in-efficient (Fig. 2). Therefore, a genotype having a small divergence from population mean can be classified as efficient/in-efficient or responsive/non-responsive if their mean is more or less than population mean. Therefore, this system may not be more helpful for screening germplasm on a large scale (Aziz *et al.* 2011, Bilal *et al.* 2018).

*Brassica* genotypes were distributed into nine groups by constructing a graph in which shoot DM was represented on the *x*-ordinate, while shoot P uptake on the *y*-ordinate. Each ordinate was dissected into three groups *i.e.*, low, medium, and high (Fig. 4). This method distinguishes the minor differences among genotypes by generating more groups (Gill *et al.* 2004; Irfan *et al.* 2017), but this system is usually applied at deficient P level (Bilal *et al.* 2018). In current study, this method showed variable results in response to with or without P addition. For instance, genotypes Con-II and Con-III rated as HDM-HP at deficient P level, grouped in MDM-MP at adequate P. Similarly, genotype Oscar initially rated as MDM-MP with P deficiency shifted to HDM-HP at adequate P level.

Some researchers classified genotypes into low, medium, and high efficient only under low P conditions (Osborne and Rengel 2002; Aziz *et al.* 2011). But this classification did not explain the performance of genotypes

under higher P availability. Since a genotype may perform well under P deficiency but may have different response with sufficient P as stated by Irfan *et al.* (2017) and Bilal *et al.* (2018). In present study, genotypes were also classified by allotting index scores as 1 for in-efficient, 2 for medium, and 3 for efficient for various parameters/indices *i.e.*, root DM, shoot DM, root-shoot ratio, total P uptake and PUE at both P supplies (Table 3). As expected, the genotypes performed differentially for each parameter under similar P environment. The genotypes Con-I, Con-II, and Con-III gained maximum index score (12 out of 15), whereas R-75/1 scored least total index (6 out of 15) under P deficiency. At adequate P level, genotype Oscar obtained maximum score (12 out of 15), whereas R-75/1 obtained lowest score (6 out of 15). According to Bilal *et al.* (2018), genotypes performing better at both P levels are more desirable for adaptation in soils having varying P contents. This classification proposed by Gill *et al.* (2004) constructs three categories of efficiency *viz.*, low, medium, and high with a substantial variation between low and high efficient genotypes allowing a broad space for medium efficient genotypes present between the two extremes (low and high). Such differences elucidate the adaptation of genotypes under diverse P availability conditions and provide the genetic basis for the execution of breeding programs.

## Conclusion

Genotypes varied with the criteria/parameters and methods used for calculating P-efficiency as some genotypes showed similar rank while other revealed divergence when categorized by different methods. Categorization of genotypes into three classes and further distribution into nine groups was the best strategy to distinguish minor differences among genotypes. Efficient genotypes *i.e.*, Oscar and Con-III performing better for DM production and P uptake at both P levels are desirable for cultivation on soils with varying P status. In addition, RDM, SDM, RSR, TPU, and PUE were important for categorizing *Brassica* genotypes for P-efficiency.

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