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Screening for Phosphorus (P) Acquisition Efficient Genotypes and Identification of Sequence Variations among P-Efficient and Inefficient Genotypes in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Low phosphorus (P) availability is the major abiotic constraint in chickpea (*Cicer arietinum* L.) cultivation across the globe as well as in India. Present study contemplated to identify high P-acquisition efficient chickpea genotypes suitable for P-deficient regions and sequence variation was detected among the tested P-acquisition efficient and inefficient genotypes. Initial screening was conducted with 104 diverse chickpea genotypes at two locations characterized by P-sufficient and deficient conditions. A panel of 14 chickpea genotypes having contrasting P-acquisition efficiency was extracted for further assessment under P-sufficient and deficient conditions. Along with the significant genetic variations, the P-efficient chickpea genotypes had high P-acquisition efficiency than P-inefficient genotypes in both the conditions. P-acquisition efficient genotypes recorded high biomass and good yield potential in P-deficient condition in comparison to P-inefficient genotypes. Sequence analysis of the *CaSPX3* domain containing protein gene from P-acquisition efficient and inefficient genotypes identified two single nucleotide polymorphisms in the 5′ - untranslated region. Present findings might be of great importance in future marker-assisted breeding in chickpea especially for P-deficient soil.

Key words: *CaSPX1* gene, Chickpea, Genetic variability, Phosphorus acquisition efficiency, Phosphorus uptake, Single nucleotide polymorphism.

INTRODUCTION

Chickpea, a cool season crop, is the world's third most widely cultivated food legume in arid and semi arid regions with excellent nutritive value in terms of human dietary proteins and livestock nutrition. In India during 2017-18, chickpea occupies 10.56 million ha area with a production of 11.23 million tonnes having the record productivity of 1063 kg/ha (Anonymous, 2018). Moreover, being a leguminous crop it has the capability of symbiotic nitrogen fixation, making this crop as a useful component of cropping system for sustaining soil health and reducing cost of cultivation for the succeeding crops. Despite of having immense potential, productivity of this legume is stagnant due to poor technological intercession and array of biotic and abiotic stresses. Additionally, narrow genetic base due to domestication from a single progenitor, *C. reticulatum* further impede genetic improvement of this crop (Abbo *et al*. 2003; Varshney *et al*. 2010).

Phosphorus (P) is an essential macronutrient for nucleic acid synthesis, membrane integrity, energy metabolism and many other critical physiological and biological processes during plant growth and development (Lambers *et al*. 2015). Legumes generally crave more P than non-legumes, as because, N_2-fixing root nodules are strong P sinks (Sprent, 1999). In the tropical and sub-tropical regions particularly in acidic and alkaline soils P is becoming one of the major limiting nutrients with slow diffusion rate and substantial fixation by other soil minerals for legume production (Krishnappa *et al*. 2011). Application of phosphatic fertilizer to overcome P deficiency is not a modest attempt due to environmental hazards and cost increment. Substantial genetic variation is available regarding various root characteristics *viz*. changes in root length, diameter, hairiness, surface area within the plant species to cope up with P-deficit situations (Gahoonia *et al*., 2006; Lynch, 2011; Haling *et al*., 2016). Keeping these in the backdrop the present study was attempted to identify the P-acquisition efficient chickpea genotypes for their further utilization in chickpea breeding programme facing low P issue as well as enumerate the association of characters with P-uptake. Moreover, attempt was made to identify the sequence

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variation among the P-acquisition efficient and P-acquisition inefficient genotypes, which could be effectively utilized for successful development of molecular marker and subsequent breeding.

MATERIALS AND METHODS

Plant materials and field experiment

Initially 104 chickpea genotypes were evaluated during winter season of 2015-16 and 2016-17 over two different locations *viz*. New alluvial soil at the District Seed Farm, 'AB' block, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, Nadia (New Alluvial Zone), West Bengal (Location 1) and Regional Research Sub Station (RRSS) of BCKV, Sekhampur, Birbhum (Red and Lateritic Zone), West Bengal (Location 2) for evaluation of their P-uptake efficiency. Finally, a subset of 14 chickpea genotypes having contrasting performance regarding P-acquisition efficiency were extracted and evaluated further at the above mentioned locations during winter season of 2017-18. The soil of the experimental field at new alluvial zone is alluvial and sandy loam in texture having good water holding capacity (WHC), medium fertility status and neutral in reaction. On contrary, the soil of the red and laterite zone has been developed from old alluvium and laterite mass which is sandy-clay loam in texture with less water holding capacity (WHC), low fertility status and acidic in reaction. Each genotype was accommodated in a row with a length of 4 m, keeping plant to plant distance of 10 cm and row to row distance of 30 cm. At the RRSS, Sekhampur, during the period of experiment, application of phosphatic fertilizer was avoided. The experiment was conducted in randomized complete block design (RCBD) with three replications. Observations were recorded as per the DUS guidelines of chickpea, on the basis of five randomly selected plants in each genotype for various yield and yield attributing traits.

Estimation of physiological parameters

For estimating the plant tissue phosphorus (P), Vanado-molybdate yellow-colour method (Jackson, 1973) was followed. Root length, shoot length, root and shoot fresh weight, root and shoot dry weight and ratio of root and shoot were recorded at 45 days after sowing. Dry weight of plant parts was estimated after drying the samples in hot air oven for 48 h maintaining at 72°C. For assessing the tolerance of P-deficiency in hydroponic experiment, seeds of 14 chickpea genotypes were first surface-sterilized following earlier protocol (Vincent, 1970). After seven days of pre-culturing period, seedlings were transferred in to new polythene covers having nutrient solution according to the method suggested by Alloush (2003). In both the nutrient solutions (High-P and Low-P), micronutrients were supplied according to the Long Ashton formula (Hewitt, 1966). For observing root behaviour pattern, seeds of P-acquisition efficient and inefficient chickpea genotypes subjected to hydroponic experiment were placed on the blotting paper and allowed to germinate in sterile water in polythene cover having glass plate. The plants were harvested after 21 days of sowing for testing the root morphology index.

DNA extraction and PCR amplification

Genomic DNA was isolated from the leaf tissues of 21 days old chickpea seedlings grown in the field. Genomic DNA was isolated using CTAB DNA extraction protocol from two PAE genotypes (IPC-2011-70 and ICCV-13318) and two P-acquisition inefficient genotypes (AGBL-146 and DCP-92-3) following earlier literature (Doyle and Doyle, 1990). For amplification of *Cicer arietinum* SPX domain-containing protein 3 genic region, forward primer CaSPX3F (5′-TACCC CTCTCACACCCCTTT-3′) and reverse primer CaSPX3R (5′-CATTCCGGCAGCGAATCTTG-3′) was used following earlier literature (Esfahani *et al*. 2016). The PCR reaction was carried out using the following thermal profile: initial denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 30 sec, 52°C for 45 sec and 72°C for 1 min and 30 sec along with a final extension at 72°C for 7 min. The PCR amplified product of four plant samples were subjected to 1% agarose gel electrophoresis along with 100 bp DNA ladder. PCR amplified products were cloned in pDrive cloning vector following manufacturer's protocol and subjected to sequence analysis.

Statistical analysis

The analysis of variance (ANOVA) was done following Gomez and Gomez (1984) and correlation coefficient was calculated by the formula suggested by Johnson *et al*. (1955) and Al- Jibouri *et al*. (1958). Significant differences among the genotypes were tested by Duncan's Multiple Range Test (Duncan, 1955) at 5% level. The statistical analysis was performed by using MS EXCEL and OPSTAT online software.

RESULTS AND DISCUSSION

Genotypic variation regarding yield attributing traits and plant tissue P-content

Analysis of variance revealed significant differences among the chickpea genotypes regarding all the 7 characters studied, thus reflected the presence of sufficient genetic variability among them in both Kalyani and Sekhampur locations (Table 1).

Mean performance of the chick pea genotypes over both the locations were represented in Table 2. It was observed that all the genotypes exhibited varied performance at both the locations. Overall, at Kalyani location the studied chickpea genotypes performed better regarding all the studied characters and P content. Overall, ICCV-13318 was the superior genotype regarding most of the yield attributing traits with almost stable performance over both the locations. Chickpea genotypes exhibited considerable variation regarding plant tissue P-content in both P-sufficient and deficient conditions. Explicitly, P-acquisition efficient chickpea genotypes had high P-acquisition efficiency than P-acquisition inefficient genotypes in low and high P condition. It was observed that plant tissue P-content of 14 chickpea genotypes ranging from 0.16 to

Table 1: ANOVA for 7 characters of 14 chickpea genotypes at Kalyani and Sekhampur locations.

Sources of variation	DF	Plant biomass (g)	No. of pod plant^{-1}	No. of seed pod^{-1}	100 seed weight (g)	P content (%)	Harvest index	Seed yield plant^{-1} (g)
Kalyani								
Replication	2	95.83	4.69	0.00	14.01	0.00	0.01	65.40
Treatment	13	27.26**	264.01**	0.10**	116.89**	0.02**	0.01**	49.90**
Environment	26	2.85	18.29	0.00	1.18	0.00	0.00	1.55
Sekhampur								
Replication	2	45.689	26.207	0.003	14.622	0	0.008	46.249
Treatment	13	38.14**	267.40**	0.06**	110.95**	0.02**	0.007**	36.3**
Environment	26	1.397	14.805	0.004	1.972	0	0	1.18

0.37% with a mean value of 0.27% in P-sufficient condition at Kalyani whereas at Sekhampur with P-deficit condition, the range was 0.13 to 0.32% with a mean value of 0.22%. Based on tissue P-content finally the 14 chickpea genotypes were classified into two categories. Genotypes *viz*. IPC-2011-70, AGBL-184, ICCV-13318, ICCV-13117, ICCV-13105, IPC-2011-123 and JG-11 with high P-acquisition efficiency was considered as P-acquisition efficient genotypes in both the conditions in comparison with the rest of the seven genotypes *viz*. DCP-92-3, FLIP-07-176, AGBL-146, FLIP-07-249C, GAG-1111, IPC-2011-69 and GJG-0904 categorized as P-acquisition inefficient genotypes. In the present study, influence of G × E was reflected in most of the studied characters. Presence of cross over interaction was confirmed by the deviation in the performance of the genotypes over both the location. However, genotype *viz*. ICCV-13318 and IPC-2011-123 revealed as consistent performers over the locations, thus confirmed the presence of non-cross over type of interaction. Therefore, in the present study within the same data set both cross over and non-cross over interaction were observed which is common over varied environments (Das *et al*. 2019a; Das *et al*. 2019b; Singh *et al*. 2020).

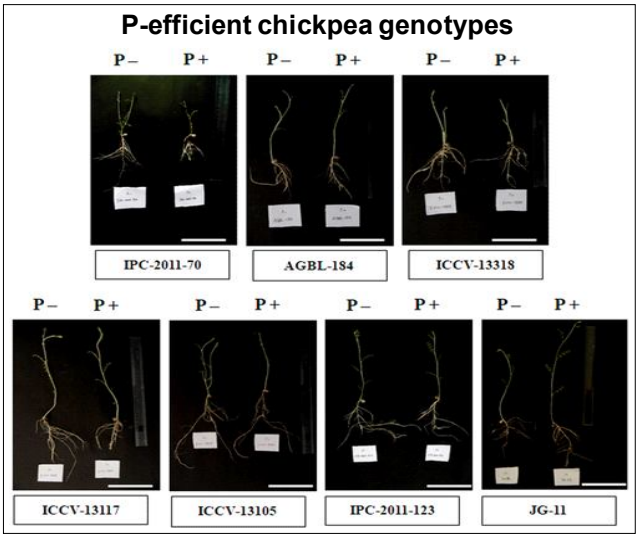

Plate 1: Root behavior of P-acquisition efficient chickpea genotypes grown in P sufficient and deficient condition.

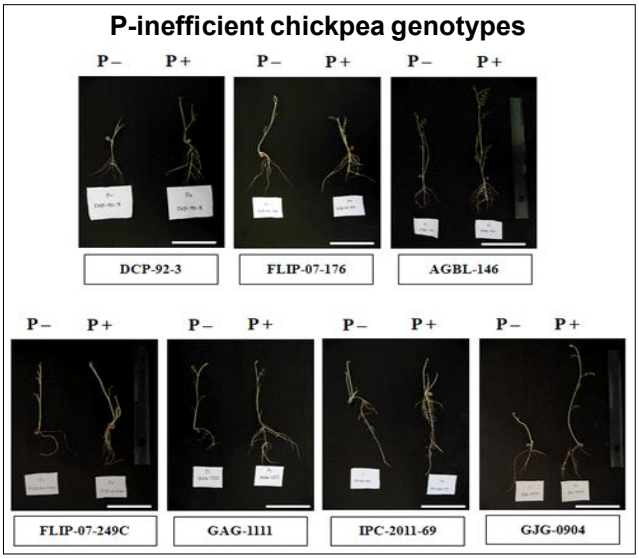

Plate 2: Root behavior of P-acquisition efficient chickpea genotypes grown in P sufficient and deficient condition.

Association of characters

Correlation studies among the 7 characters indicated different degree of association between the characters at genotypic and phenotypic levels (Table 3). In the present study, it was observed that genotypic correlation coefficients were of higher magnitude than the phenotypic correlation coefficients at both the locations. At Kalyani location, seed yield plant^{-1} showed significant and positive association with number of pods plant^{-1}, number of seeds pod^{-1}, plant biomass, harvest index and P-content at both genotypic and phenotypic levels which suggested that selection for seed yield plant^{-1} based on these traits would be beneficial. At Sekhampur location, seed yield plant^{-1} revealed significant and positive association with all most all the above mentioned characters except number of seeds pod^{-1}. In harmony with the present finding the significant and positive

Table 2: Mean performance of 14 chickpea genotypes for 7 yield attributing characters and P-content at Kalyani (Loc.1) and Sekhampur (Loc. 2) location.

Genotype	Plant biomass (g)		No. of pod plant^{-1}		No. of seed pod^{-1}		100 seed weight (g)		P content (%)		Harvest index		Seed yield plant^{-1} (g)	
	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2
IPC-2011-70	34.05^{bc}	31.21^{abc}	57.17^{a}	46.40^{a}	1.49^{bc}	1.33^{cd}	17.91^{g}	17.70^{f}	0.37^{a}	0.32^{a}	0.22^{bc}	0.20^{cd}	13.26^{c}	11.15^{b}
AGBL-184	33.73^{bcd}	32.20^{ab}	49.97^{abc}	43.92^{a}	1.53^{ab}	1.35^{cd}	25.74^{de}	24.09^{de}	0.36^{ab}	0.31^{a}	0.26^{a}	0.22^{abc}	17.06^{ab}	13.03^{ab}
ICCV-13318	36.50^{ab}	32.48^{a}	46.00^{bcd}	31.63^{b}	1.30^{ef}	1.55^{a}	37.70^{a}	36.05^{a}	0.35^{ab}	0.31^{a}	0.26^{a}	0.24^{ab}	19.26^{a}	14.71^{a}
ICCV-13117	33.53^{bcd}	30.45^{abc}	48.15^{bc}	42.92^{a}	1.42^{cd}	1.37^{cd}	24.28^{ef}	23.05^{de}	0.34^{ab}	0.27^{b}	0.23^{ab}	0.20^{cd}	14.87^{bc}	11.13^{b}
ICCV-13105	35.15^{ab}	29.72^{c}	52.43^{ab}	48.35^{a}	1.60^{a}	1.35^{cd}	22.93^{f}	22.03^{e}	0.35^{ab}	0.30^{ab}	0.24^{ab}	0.22^{abc}	16.07^{b}	12.24^{b}
IPC-2011-123	37.65^{a}	31.64^{abc}	53.50^{ab}	49.00^{a}	1.55^{ab}	1.30^{cd}	26.39^{d}	25.41^{d}	0.36^{ab}	0.30^{ab}	0.25^{a}	0.24^{a}	19.18^{a}	14.88^{a}
JG-11	33.75^{bcd}	30.05^{bc}	53.40^{ab}	48.40^{a}	1.58^{a}	1.40^{bc}	22.62^{f}	21.66^{e}	0.33^{b}	0.29^{ab}	0.25^{a}	0.22^{bc}	16.35^{b}	11.89^{b}
DCP-92-3	29.50^{e}	25.35^{d}	52.80^{ab}	32.42^{b}	1.35^{de}	1.50^{ab}	13.22^{h}	12.96^{g}	0.18^{de}	0.13^{de}	0.18^{d}	0.14^{h}	8.90^{de}	5.68^{d}
AGBL-146	30.66^{de}	25.86^{d}	45.31b^{cd}	29.52^{bc}	1.25^{f}	1.50^{ab}	17.81^{g}	17.04^{f}	0.24^{c}	0.14^{cde}	0.18^{d}	0.15^{fgh}	9.28^{de}	6.38^{cd}
FLIP-07-176	31.07^{cde}	24.54^{d}	38.26^{de}	28.88^{bc}	1.00^{h}	1.00^{f}	26.93^{b}	27.49^{c}	0.21^{cd}	0.17^{c}	0.19^{cd}	0.18^{de}	10.80^{d}	8.06^{c}
FLIP-07-249C	28.85^{e}	24.58^{d}	31.45^{ef}	25.45^{bc}	1.10^{g}	1.17^{e}	31.64^{b}	31.51^{b}	0.16^{e}	0.11^{e}	0.19^{cd}	0.17^{ef}	9.28^{de}	7.12^{cd}
GAG-1111	30.77^{de}	25.02^{d}	43.22^{cd}	29.77^{bc}	1.27e^{f}	1.32^{cd}	19.46^{g}	17.99^{f}	0.21^{cd}	0.16^{cd}	0.19^{cd}	0.17^{efg}	10.50^{de}	7.05^{cd}
IPC-2011-69	29.05^{e}	24.64^{d}	33.35^{ef}	29.83^{bc}	1.32^{ef}	1.25^{de}	24.09^{ef}	22.39^{de}	0.21^{cd}	0.16^{cd}	0.17^{d}	0.14^{gh}	8.48^{de}	5.66^{d}
GJG-0904	28.04^{e}	22.02^{e}	26.73^{f}	23.57^{c}	1.35d^{e}	1.27^{de}	29.38^{c}	27.70^{c}	0.22c^{d}	0.15^{cd}	0.17^{d}	0.15^{fgh}	8.29^{e}	5.27^{d}

Different letters indicate significant difference at P<0.05 (or means within columns separated by Duncan's multiple range test P=0.05; Duncan, 1955).

Table 3: Below diagonal (Genotypic) and above diagonal (Phenotypic) representation of correlation coefficients between yield and its component characters in chickpea at Kalyani (Loc.1) and Sekhampur (Loc. 2) location.

	No. of pod plant^{-1}		No. of seed pod^{-1}		100 seed weight (g)		Plant biomass (g)		P content (%)		Harvest index		Seed yield plant^{-1} (g)	
	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2	Loc. 1	Loc. 2
No. of pod plant^{-1}	**1.000**	**1.000**	0.615**	0.184NS	-0.438**	-0.232	0.597**	0.778**	0.659**	0.794**	0.573**	0.649**	0.591**	0.710**
No. of seed pod^{-1}	0.694**	0.250	**1.000**	**1.000**	-0.215	-0.249	0.533**	0.413**	0.725**	0.262	0.569**	0.075	0.594**	0.231
100 seed weight (g)	-0.475**	-0.248	-0.235NS	-0.290	**1.000**	**1.000**	0.185	0.125	0.121	0.137	0.316*	0.377*	0.342*	0.324*
Plant biomass (g)	0.807**	0.836**	0.638**	0.439**	0.227	0.111	**1.000**	**1.000**	0.793**	0.900**	0.673**	0.796**	0.830**	0.905**
P content (%)	0.732**	0.880**	0.780**	0.317*	0.123	0.147	0.967**	0.961**	**1.000**	**1.000**	0.817**	0.842**	0.844**	0.892**
Harvest index	0.658**	0.776**	0.638**	0.124	0.363*	0.411**	0.964**	0.915**	0.936**	0.908**	**1.000**	**1.000**	0.956**	0.962**
Seed yield plant^{-1} (g)	0.656**	0.797**	0.622**	0.268	0.358*	0.338*	0.976**	0.964**	0.910**	0.949**	0.098	0.989**	**1.000**	**1.000**

association for number of pod plant^{-1}, plant biomass, 100 seed weight and harvest index were reported in earlier studies (Aarif *et al*. 2013; Jagadish and Jayalakshmi 2015; Chopdar *et al.* 2017).

Effect of P on physiological parameters

In hydroponic study considerable genetic variation was exhibited regarding root and shoot morphological parameters. P-acquisition efficient genotypes reflected better tolerance and genetic homeostasis under P-deficit condition (Plate 1 and 2). It was observed that among the P-acquisition efficient genotypes, ICCV-13105 recorded the highest root length (17 cm) followed by JG-11 (13.5 cm) in high P-level (Fig 1a). JG-11 and GJG-0904 from P-acquisition efficient and inefficient groups respectively recorded highest shoot length in high P-condition (Fig 3a). The root to shoot ratio of chickpea genotypes increased by 2.64 ~ 18.84% in low P-treatment compared to high P-situation (Fig 1b). Root fresh weight of chickpea genotypes reduced by 9.62-33.3% in low P-condition compared to high P-condition whereas regarding shoot fresh weight the reduction rate was 11.19-31.16 % (Fig 2a). On contrary in case of inefficient genotypes the reduction rate was 26.85% and 31.47% respectively. Like root and shoot fresh weight, same trend was exhibited in case of root and shoot dry weight also (Fig 2b). Therefore, it can be deduced that in low P-condition the performance of P-acquisition efficient genotypes were better regarding root and shoot morphological parameters than the inefficient genotypes though both the genotypes groups revealed better performance in P-sufficient situation. Previous reports confirmed the role of root physiological adaptation towards improvement in P availability as well as further P-use efficiency (Pang *et al*. 2010; Shen *et al*. 2011; Zhou *et al*. 2016).

Sequence analysis among the P-acquisition efficient and inefficient genotypes

Finally, among these high P-acquisition genotypes, ICCV-13318 and IPC-2011-70 were considered for high biomass yield and high P-content respectively and subsequently used for further molecular analysis. On the other hand, two genotypes *viz*. AGBL-146 and DCP-92-3 belonging to low P-category were considered for further molecular analysis due to maximum reduction in P-content in low P containing

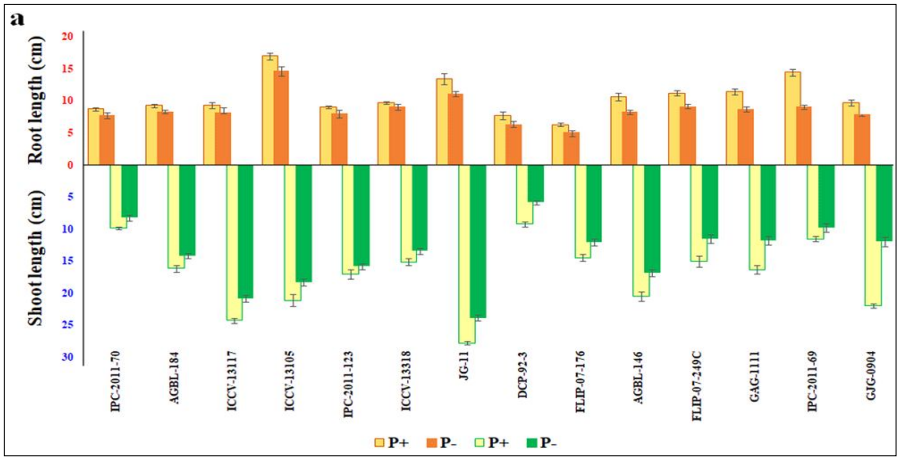

Fig 1a: Root and shoot length (cm) of 14 chickpea genotypes under P sufficient and deficient condition.

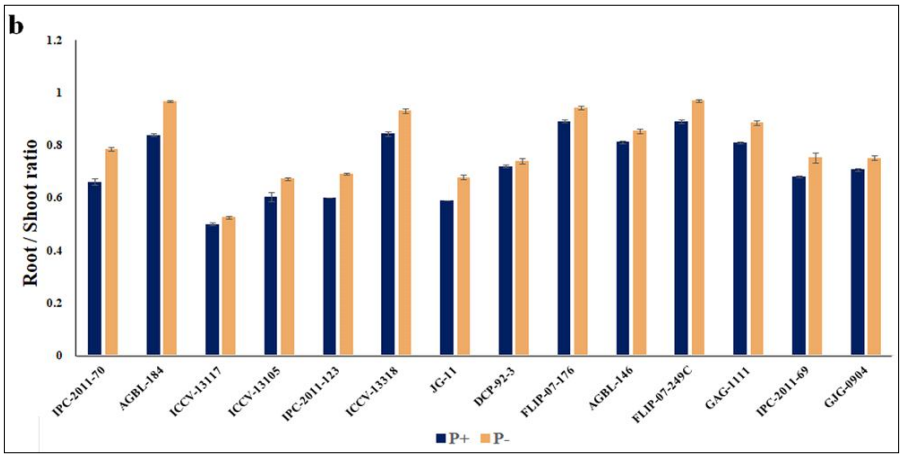

Fig 1b: Root: shoot ratio of 14 chickpea genotypes under P sufficient and deficit condition.

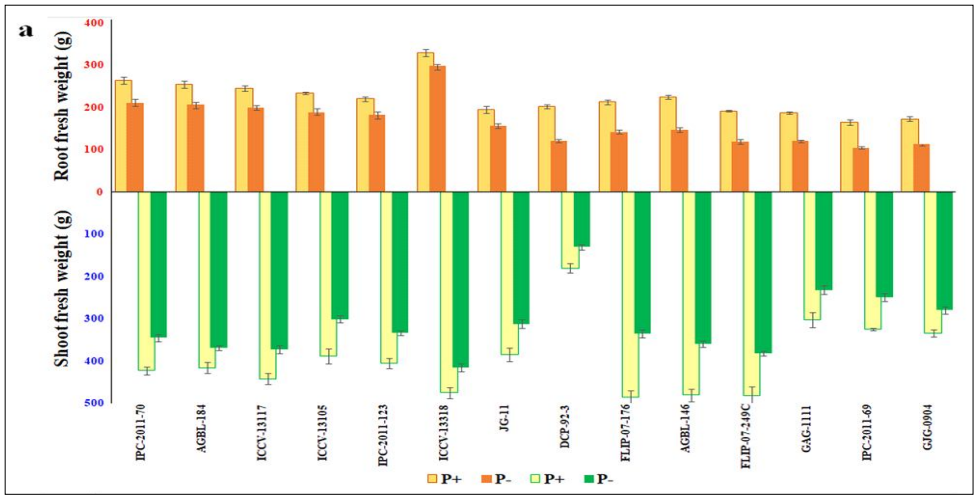

Fig 2a: Root and shoot fresh weight (g) of 14 chickpea genotypes under P sufficient and deficient condition.

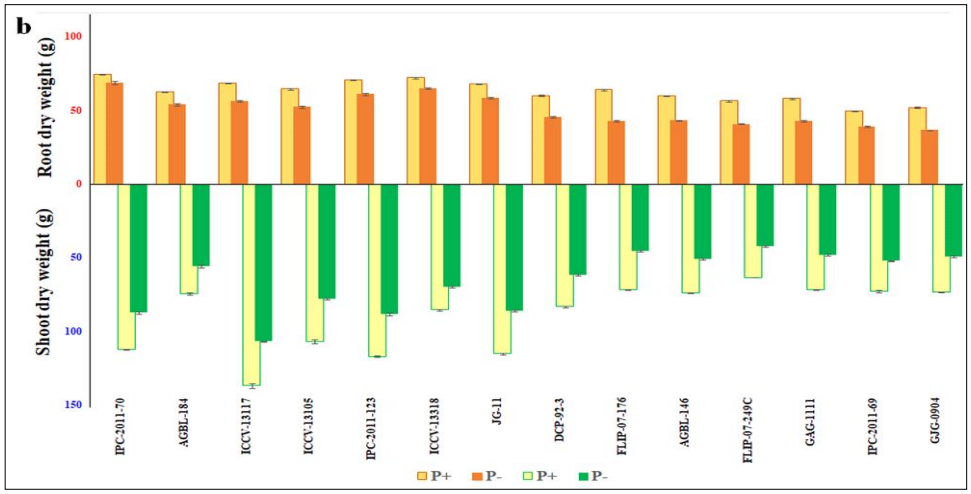

Fig 2b: Root and shoot dry weight (g) of 14 chickpea genotypes under P sufficient and deficient condition.

soil and due to low P-content and Harvest Index respectively across two locations.

DNA amplification of two P-acquisition efficient chickpea genotypes (IPC-2011-70 and ICCV-13318) and two P-acquisition inefficient chickpea genotypes (AGBL-146 and DCP-92-3) through polymerase chain reaction (PCR) using CaSPX3F and CaSPX3R depicted an amplified product of ~190 bp in all of the genotypes. Sequence analysis of the amplified region through Basic Local Alignment Search Tool (BLAST) was found to hit at the 5′-untranslated (5′-UTR) region and part of *Cicer arietinum* SPX domain-containing protein 3 (*CaSPX3*) mRNA at National Centre for Biotechnology Information (NCBI) database. In addition to that two single nucleotide polymorphisms (SNP) were detected among the P-acquisition efficient and inefficient chickpea genotypes. One SNP was identified at the 4th base of the 5′-UTR and that was120 nucleotide (nt) upstream from the start codon and another SNP was detected at 25 nt upstream from the start codon of *CaSPX3* gene (Fig 3).

For further confirmation of the available SNP, in *Cicer arietinum* SPX domain-containing protein 3 genic region, re-sequencing was done using CaSPX3R primer and that depicted an adenine (A) residue in two P-acquisition efficient chickpea genotypes (IPC-2011-70 and ICCV-13318) while in two P-acquisition inefficient chickpea genotypes (AGBL-146 and DCP-92-3), a guanine (G) residue was available at 120 nt upstream from the start codon (Fig 4). Additionally, at 25 nt upstream from the start codon of CaSPX3 gene, an 'A' residue was found to be available in two P-acquisition efficient chickpea genotypes while in two P-acquisition inefficient chickpea genotypes cytosine (C) residue was detected at that position.

It is to be recalled here that the SNP detected in the present study is available in the 5′ -UTR region of a gene (CaSPX3) involved in phosphorus signalling network (Esfahani *et al*. 2017) and this gene might play a crucial role in phosphorous homeostasis. Moreover, a recent study revealed that the RNA structure at 5′ -UTR region controls plant gene expression involving microRNA (Gu *et al*. 2014).

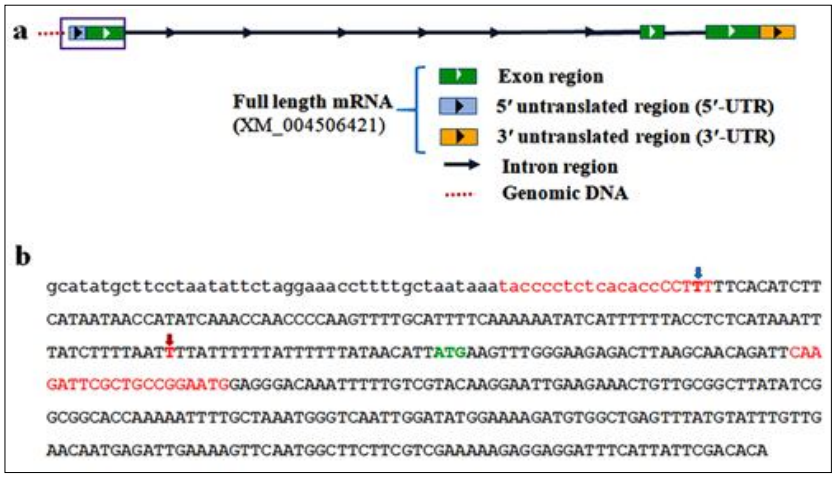

Fig 3: *Cicer arietinum* SPX domain containing protein 3 gene (*CaSPX3*) structure. (a) Illustration of the locus (LOC101498771) corresponding to *CaSPX3* gene along with the mRNA, intron and some of the upstream genomic DNA region. (b) The sequence of the purple boxed region of (a) is shown. The genomic DNA sequences are in lowercase and the mRNA sequences are in uppercase. The start codon is shown in green color and two SNP identified are shown by brown arrow (at 25 nucleotide upstream from start codon) and blue arrow (at 120 nucleotide upstream from start codon). Two primers used for PCR amplification of *CaSPX3* gene are shown in red color.

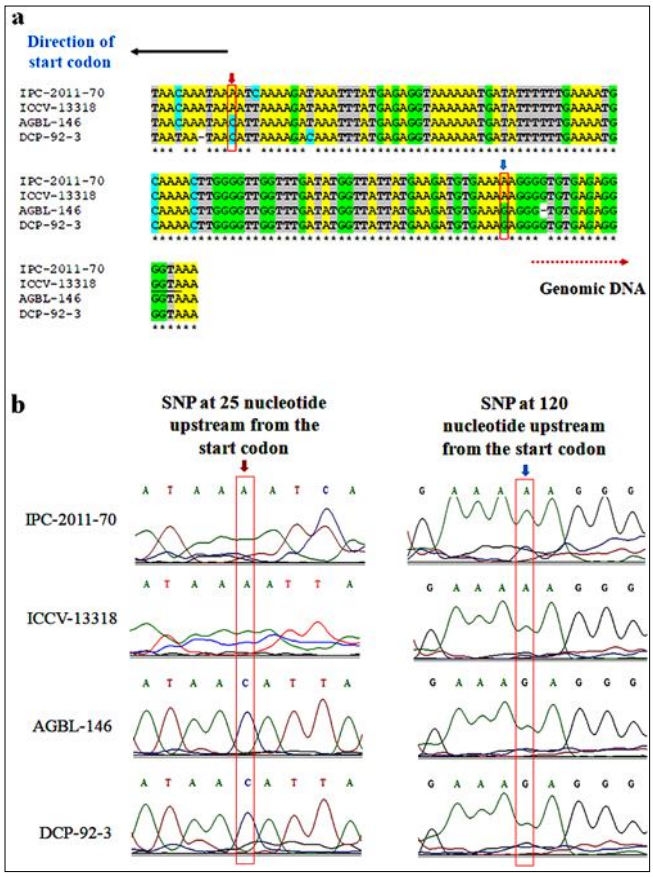

Fig 4: Multiple sequence analysis of two P-acquisition efficient and two P-acquisition inefficient chickpea genotypes and identification of single nucleotide polymorphism (SNP). (a) Multiple sequence analysis of two P-acquisition efficient (IPC-2011-70 and ICCV-13318) and two P-acquisition inefficient (AGBL-146 and DCP-92-3) chickpea genotypes by CLUSTALW. CaSPX3 gene was sequenced using CaSPX3R primer from P-acquisition efficient and P-acquisition inefficient genotypes. Two SNPs are shown in red boxes with brown arrow (at 25 nucleotide upstream from start codon) and blue arrow (at 120 nucleotide upstream from start codon). The direction of start codon and genomic DNA region are shown by black arrow and dotted red arrow, respectively. (b) Part of the sequence file showing the indicated SNPs in different chickpea genotypes.

Hence a minor modification in the 5′ -UTR of CaSPX3 might change the phosphorus homeostasis in plants. Further study is needed to understand the involvement of this available SNPs in expression of *CaSPX3* gene at transcript as well as protein level. Further screening is needed to identify this sequence variation as a potent molecular marker associated with P-acquisition in chickpea.

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