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Screening of Common Bean (*Phaseolus vulgaris* L) Germplasm for Drought Stress

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Common bean is a species having high nutritional composition. Drought response in plants is complex phenomenon relating interactions between structure, functions and development of the plant. The Improvement of drought resistant for common bean has major objectives for many breeding programs. The growth of the plant, related to the parameter plays a vital role in the selection criteria for drought resistance. The increasing drought tolerance in commercial varieties is highly desirable. 20 varieties of common bean leaves were collected, it is subjected in *vitro* water stress with 10% polyethylene glycol 6000 (PEG 6000). The parameters are determined as a relative water content (RWC), seedling root length, leaf area, stomatal index, wax and proline. The result points out that these varieties responded differently during the drought stress. Tolerant and Susceptible comes under the varieties that are categorized which are based on RWC. The tolerant varieties maintain a high water content. Generally, the tolerant varieties are higher in seedling root length, leaf area, stomatal index, wax than the susceptible varieties. The proline accumulations are higher in susceptible varieties when compared to the tolerant varieties. A significant correlation was observed from the above parameters. The results are discussed with reference to the plant's response during drought.

Key words: Common bean, Drought, Proline, Relative water content, Stomatal index, Susceptible, Tolerant, Wax

The common bean seeds (*Phaseolus vulgaris* L) are rich in proteins, carbohydrates, fibers and minerals which makes a good source of nutrients (Polo, 1993). Droughts improvement of drought resistance in common bean is a major objective for many breeding programs (Migul *et al*., 2012). Drought resistances in genotypes are associated with different kinds of mechanisms such as morphological and physiological functions which maintain the growth of the plant. It is expected that plants will show an increased tolerance to drought which has the capacity to protect their conductive tissue (Victor *et al*., 2008). Blum (1988) has reported the drought susceptibility of a genotype, often measured as function of the reduction that yields under drought stress.

Relative water Content (RWC), is used as key indicator of the degree cell and tissue hydration, that is crucial when the optimum physiological functioning and growth processes is taking place (Zadraznik *et al.,* 2012). RWC, an important determinant of survival of leaves and metabolic activity (Sinclair and Ludlow, 1986) it is an attribute for discriminating drought tolerant and sensitive genotypes (Rauf, 2008). The tolerant genotypes maintained higher water status in leaves than the susceptible genotypes (Choudhury *et al.* 2014; Mokter *et al*., 2014). Rooting depth is an important component in determining overall drought tolerant in beans and in root architecture characters no relationship between number of basal whorls and drought tolerance (Lydia *et al.,* 2013). White and Castilo (1989) believe that root architecture is of primary importance in determining and developing drought tolerant in common bean.

Leaf area is the most important morphological adaptation (Acosta-Gallegos, 1988). Generally the tolerant genotypes maintained a greater leaf area rate than the susceptible genotypes (Makter *et al*., 2014). The drought tolerant species reduce the water loss either by reducing leaf area in less effect on the biomass production (Lazaridou *et al*., 2003). The tolerant genotype was characterized by a higher percentage of stomata and a wider stomata pore diameter than the susceptible genotype (Trived, 2014). Despite the mechanism of drought tolerance, a canopy that is able to use more water has more open stomata, a higher depression of canopy temperature and higher C-13 discrimination in plant matter (Araus *et al*., 2002).

Proline accumulation is one of the most frequently reported water stress induced biochemical response in plants. It's often considered to be involved in stress resistance mechanisms (Kavi *et al.,* 1995). Some studies have suggested that proline accumulates in drought susceptible cultivars than the tolerant varieties (Sanchez *et al.*, 2010). These traits are also relevant when breeding plants for hot, irrigated environments (Araus *et al.* 2002). Leaf epicuticular wax content decreased net radiation in the field, and decreased culticular transpiration. Epicuticular wax is an effective component of drought resistance (avoidance mechanisms in Sorgum) (Ebercon *et al*., 1977). Conditions of water stress, as may have developed in this dryland study, were found to promote epiculticular content (Skoss, 1955).

This study is carried out with 20 different varieties of common bean, which were measured using parameters such as root architecture, leaf area, stomatal index, proline, and major drought stress inducing 10% of PEG for relative water content.

MATERIALS AND METHODS

The trifoliate leaves *in vitro* water stress induced 10% of PEG 6000 at 6 Hrs. Leaf relative water content was estimated by a following equation RWC(%)= (FW-DW)/TW- DW*100, whereas (FW- fresh weight, DW- oven dry weight at 37°C for 24 Hrs and TW- Turgid Weight) of the leaflets (Smart and Binglam, 1974). Seeds washed with 1% of Tween 20 on the germinate sheets and 2% of kanamycin acid sulphate antibiotic agents were added and after 10 days root architecture characters were measured (Mcgee, 1988; Kaydan and Yagumur, 2008).

The leaves were selected randomly in each variety and leaves were spread over the square centimeter graph paper and outline of leaf was drawn. The leaf area calculated in each square centimeter (Pandey and Singh, 2011). To study the stomatal index, fresh leaves were directly fixed into the fixative FAA (Formalin, Acetic acid and Alcohol in the ratio 1:1:18 respectively). The epidermial layers (adaxial and abaxial surface) were peeled and placed is 30% Hydrogen peroxide, later it is stained in 1% of saffrain and finally mounted in glycerin. The stomata and frequency distributions were formulated using the stomatal Index (SI) thus designated as SI = No. of Stomatal (S) /No. of epidermal cells (E)+S*100 (number of stomata per mm) (Edwin *et al.,* 2012).

Free proline levels were determined using the ninhydrin reaction according to the method of Bates *et al.* (1973; Irigoyen et al 1992). Proline concentration was determined from a proline standard curve. Freshly harvested was immersed in 15ml of redistilled chloroform and allowed 20s (only the epicuticular wax will be extracted into chloroform, a longer time may extract the inner lipids), the chloroform extract is boiled on water bath until the smell of chloroform goes off completely. About 5ml of wax reagent (20gm of potassium dichromate with 40ml of deionzed water the resilting slrry is then mixing with 1 lit of H_2SO_4 (concentrated) is added and boiled for about 30min in water bath. The boiled sample is cooled and 12ml of deionized water is added. After cooling, the extract is filtred using the filter paper and filtred was collected. The indesity of color is determined using spectrophotometer at 590nm (Mamrutha et al., 2010). Finally, statistical analysis was done by using spss software version 20.

RESULTS

Drought tolerant parameters such as RWC, seedling root architecture characters, leaf area, stomatal index and proline were measured and the mean and standard deviation in tolerant and susceptible varieties were calculated. The Pearson correlations were observed in the above parameters with all the 20 varieties of common bean (Table 3). Among the 20 different varieties of common bean, tolerant and susceptible varieties were categorized based on RWC in 10% PEG 6000. The tolerant varieties have high water holding capacity (68-62%) when compared to susceptible varieties (49-60%) in 6 hours stress. The tolerant varieties are LR 4, 1, 15, 12, 8, 9, 2

and susceptible varieties are LR 14, 6, 18, 5, 16, 19, 20 respectively (Table 1).

The root architecture character traits of the tolerant and susceptible varieties give different values. The tolerant varieties show the deep tap root length 16cm in LR 2 where the susceptible varieties show 5cm in LR 5. The numbers of basal root is higher in tolerant varieties 40 in LR 1 and lower 1 LR 4. In susceptible varieties the number of basal root is higher 40 in LR 19 and lower 24 in LR 16. Similarly the tolerant varieties have higher number of root whorls with an average of 5.2 in LR 12 and lower 2.5 in LR 2. The susceptible varieties have highest number of root whorls with an average 5.16 in LR 18 and lowest 3.5 in LR 6 and 19. The tolerant varieties have higher number of basal root whorls with an average of 3.75 in LR 9 than the susceptible varieties with an average 2 in LR 16 (Table 2). The leaf area in tolerant varieties have high leaf surface area 84.5 cm^2 in LR 8 and lower leaf surface area 54 cm^2 in LR 2 and LR 5. In susceptible varieties higher leaf surface area is about 105.5 cm^2 in LR 5 and lower leaf surface area is about 45.1 cm^2 in LR 18 (Table 2). Different values were observed between tolerant and susceptible varieties based on leaf area. The stomata types are observed in 20 different varieties of common bean. There are two types of stomata (anomocytic and anisocytic) based on their structure(Fig 2). Generally anomocytic stomata that are found abundance in both tolerant and susceptible varieties. In tolerant varieties, the highest stomatal index of both adaxial and abaxial surface is 25.8mm^2 (LR 15) and 34.9 mm^2 (LR 2) respectively. Similarly the lowest stomatal index of adaxial and abaxial surface is 13.2mm^2 (LR 9) and 22.9 mm^2 (LR 4) respectively. In susceptible varieties, the highest stomatal index of both adaxial and abaxial surface is 21.7mm^2 (LR 6) and 33.8 mm^2 (LR 6) respectively. Similarly the lowest stomatal index of adaxial and abaxial surface is 5.32mm^2 (LR 16) and 13.1 mm^2 (LR 14) respectively (Table 2).

The proline accumulations are observed while it is treated with 10% PEG 6000 at 6 hours stress. The susceptible varieties have higher proline content than the tolerant varieties. The higher proline content is 2.13 µM/g tissues in LR 18 and lower proline content is 0.62 µM/g tissue in LR 9 (Table 2). The positive correlation which has been observed between all the 20 different varieties in all the parameters such as RWC, seedling root architecture characters, leaf area, stomatal index and proline are given in the illustrated table (Table 2). Different value that is observed between tolerant and susceptible varieties are based on RWC, tap root length, leaf area, abaxial surface of stomatal index and proline as given in the figure (Fig 1). The leaf surface wax amount on these 20 varieties from minimum 25.64µg dm^{-2} to a maximum of 367.52 µg dm^{-2}. The high wax amount contains LR 4- 367.52 µg dm^{-2}, LR 10 -358.77 µg dm^{-2} and LR 2- 307.69 µg dm^{-2} and low wax amount contain LR 1 and LR 18- 25.64µg dm^{-2}, and LR 6- 34.1864 µg dm^{-2}. (Table 2).

The positive correlation observed between the characterizations of 14 varieties in common bean with all the parameters such as RWC, seedling root characters, leaf area, stomatal index, wax, and proline are mentioned in the table (Table 2). Different values that are observed between tolerant and susceptible varieities are based on RWC; tap root length, leaf area abaxial surface of stomatal index, wax and proline as illustrated in the figure (Fig 1 & Fig 2).

Table.1: Relative Water Content (RWC) in 20 Landraces of common bean subjected to *invitro* water stress with 10% Polyethylene glycol 6000 (PEG)

S.No	Landraces	RWC (%)			Categorized Genotypes		
		2 Hrs	4 Hrs	6 Hrs	Landraces	6 Hrs	Categorized Genotype
1	**LR 1**	**81**	**72**	**67**	**LR 4**	**68**	**Tolerant**
2	**LR 2**	**82**	**73**	**66**	LR14	49	Susceptible
3	LR 3	80	66	51	**LR 1**	**67**	**Tolerant**
4	**LR 4**	**87**	**76**	**68**	LR 6	58	Susceptible
5	LR 5	76	64	57	**LR15**	**66**	**Tolerant**
6	LR 6	80	68	58	LR 18	50	Susceptible
7	LR 7	74	62	51	**LR12**	**65**	**Tolerant**
8	**LR 8**	**85**	**67**	**64**	LR 5	57	Susceptible
9	**LR 9**	**79**	**67**	**62**	**LR 8**	**64**	**Tolerant**
10	LR 10	80	67	56	LR16	57	Susceptible
11	LR 11	81	63	54	**LR 9**	**62**	**Tolerant**
12	**LR12**	**79**	**71**	**65**	LR19	55	Susceptible
13	LR 13	81	67	51	**LR 2**	**66**	**Tolerant**
14	LR14	75	58	49	LR20	60	Susceptible
15	**LR15**	**82**	**73**	**66**			
16	LR16	88	73	57			
17	LR 17	80	69	51			
18	LR 18	80	61	50			
19	LR19	83	69	55			
20	LR 20	81	70	60			

Table.2: Root architecture characters of tolerant and susceptible in common bean varieties

S. N o	Landr aces	Categorized Genotype	Root System (cm)				Leaf area (cm)^2	Wax (µg dm^{-2})	Stomatal Index (mm)^2		Proline (µM/g)	
			Tap Root Length	No. Basal Root	No. Root Whorls	No. Basal Root Whorls			Adaxial	Abaxial	Control	Test
1	**LR 4**	**Tolerant**	**6.66±1.75**	**1.66±0.81**	**3.83±1.16**	**2.75±0.95**	**75.8±3.43**	**367.5±4.7**	**16.6±0.91**	**22.9±0.59**	**0.75±0.8**	**1.24±1.1**
2	LR14	Susceptible	5.16±1.8	24.6±4.08	4.16±1.16	3.75±0.5	68.6±9.2	68.5±4.8	5.6±0.7	13.1±1.01	1.78±0.04	2.06±0.09
3	**LR 1**	**Tolerant**	**13.3±2.33**	**40.6±3.52**	**4.16±1.16**	**3.5±0.5**	**64.2±8.75**	**25.6±3.8**	**15.6±0.54**	**26.3±0.91**	**1.05±0.03**	**1.5±0.22**
4	LR 6	Susceptible	14.1±0.98	32.5±3.39	3.5±1.37	2.25±1.25	60.5±5.3	34.2±4.7	21.7±0.38	33.8±0.64	1.29±0.06	1.34±0.03
5	**LR15**	**Tolerant**	**9.83±3.76**	**15.5±2.88**	**4.5±1.04**	**2.25±0.5**	**54.5±8.2**	**34.2±4.3**	**25.8±0.54**	**25.3±0.86**	**1.07±0.05**	**1.34±0.07**
6	LR 18	Susceptible	10.8±0.75	25.8±4.87	5.16±0.75	3.4±1.51	45.1±8.4	25.6±3.3	21.5±1.1	32.1±0.73	1.04±0.10	2.13±0.06
7	**LR12**	**Tolerant**	**8.16±1.16**	**28.5±2.25**	**5.2±0.75**	**3.5±1.29**	**73.6±7.47**	**102.6±7.2**	**19.2±0.95**	**24.4±1.21**	**0.89±0.06**	**1.44±0.06**
8	LR 5	Susceptible	5.3±1.03	28.8±2.92	3.66±1.36	3±0.81	105.5±5.5	52.2±4.2	18.1±0.78	22.8±0.66	1.04±0.02	1.76±0.06
9	**LR 8**	**Tolerant**	**15±1.41**	**38.3±5.04**	**4.5±1.04**	**3±0.81**	**84.5±7.0**	**59.9±4.6**	**18.5±0.98**	**30.3±0.62**	**0.65±0.05**	**1.12±0.09**
10	LR16	Susceptible	14.8±1.47	24.1±4.02	4±1.41	2±0.71	74.1±4.71	42.8±3.7	5.32±0.92	28.1±0.54	0.97±0.05	1.33±0.04
11	**LR 9**	**Tolerant**	**6.16±1.60**	**40.6±3.72**	**5±0.89**	**3.75±0.95**	**71.6±6.86**	**222.2±5.8**	**13.2±0.73**	**26.4±0.57**	**0.62±0.08**	**1.12±0.09**
12	LR19	Susceptible	15.1±1.94	40.5±3.61	3.5±1.64	2.2±0.83	73.8±6.08	192.3±9.8	12.1±0.50	32.1±1.00	0.97±0.03	1.78±0.10
13	**LR 2**	**Tolerant**	**16.3±1.21**	**29.6±3.98**	**2.5±1.64**	**2.25±0.5**	**54.2±8.49**	**307.7±4.1**	**18.4±0.98**	**34.9±1.44**	**0.95±0.03**	**1.05±0.02**
14	LR20	Susceptible	14.6±1.75	39.3±4.71	5.33±0.81	3.8±0.83	60.1±11.1	111.1±7.3	18.4±0.45	28.5±0.72	0.69±0.11	1.09±0.06

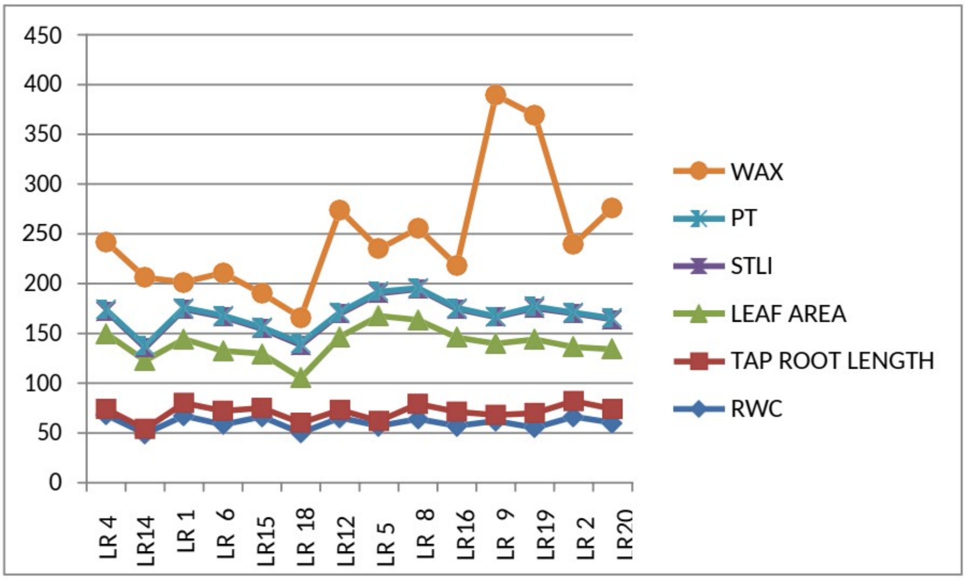
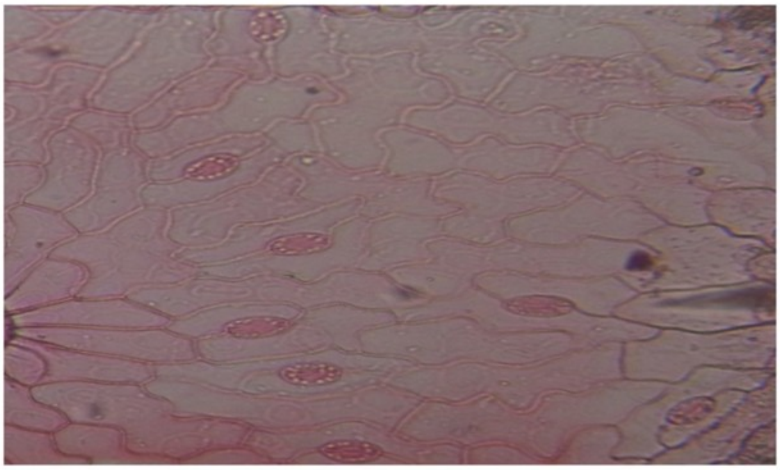


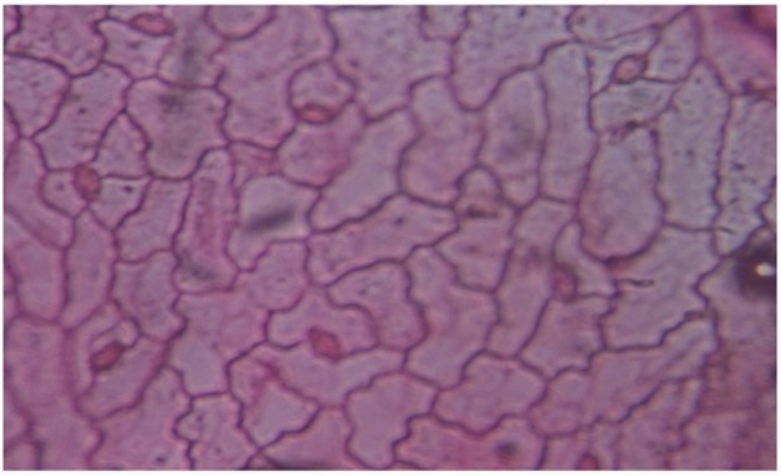
Figure 1 Graph Differences between tolerant and susceptible varieties of common bean.

Table.2: Pearson Correlations between the 20 varieties of common bean.

	RWC 4 Hrs	RWC 6Hrs	Basal whorls	Stomatal abaxial	Proline Test	Wax
RWC 2 Hrs	.715**	.464*				
RWC 4 Hrs		.765**				
Tap Root				.688**		
Whorls			.813**			
Proline Control					.736**	-445**



Stomatal Type- Anomocytic



Stomatal Type- Anisocytic

Figure 2 Difference between Stomatal Types

DISCUSSION

Common bean is cultivated under drought condition in several developing countries, where drought is the most important factor that limits the production of Latin America, Brazil, Mexico and Africa. They have evolved several mechanisms to maintain plant, water status within reasonable limits for normal metabolic functioning under the drought stress (Beebe, 2012). The common bean water stress reduced between 19% and 37% in the first trifoliate leaf (Stoyanov, 2015) the RWC maintaining capacities in cultivated varieties were more productive (Kumar and Sharma, 2013) and percentage of RWC of excised leaves of the susceptible genotype was substantially lower than that tolerant genotypes and that genotypes from the plant, can retain higher % RWC in water-limited environments (Mokter, 2014). The water stress conditions and the tolerant varieties maintenance of a high RWC (Choudhury *et al.* 2014; Clarke and Mccaige 1982; Ritches *et al* 1990). According to our results the reduced water stress between 14% and 31% in the terminal leaf and the tolerant varieties have higher water holding capacity (68%-62%) compared to susceptible varieties (49%-60%) in 6 hrs stress. The tolerance varieties maintaining higher water content than the susceptible varieties.

Drought tolerant varieties have longer root length and highest root weight than susceptible varieties (Lydia *et al*., 2013), drought avoidance through greater root length density and deeper soil moisture (Sponchiado *et al.*, 1989). Generally root length was higher in the tolerant genotypes than the susceptible genotypes. The results of the present study indicate that the deep tap root length of the tolerant variety is higher (16cm in LR2) than the susceptible variety which is over (5cm in LR5). Likewise, basal root shows higher value than the susceptible one. Also, the tolerant varieties have higher number of root whorls than the susceptible varieties.

A significantly decrease in leaf area of the susceptible parents could be due to an accelerated leaf senescence as well as loss of turgor (Canvar *et al*., 2014). In the cases of stress and non-stress conditions, leaf area in susceptible genotypes was more than that in tolerant genotypes, thus drought tolerance may be attributed to less transpiration and water loss because of smaller size and reduced leaf area in tolerant genotypes when drought stress develop (Ganjaela, 2011). The results shows that higher leaf surface area of 105.5 cm^2 in LR 5 which is one of the susceptible variety than the tolerant variety showing leaf surface area of 84.5 cm^2 in LR 8. The number of stomata between the abaxial surface and the adaxial surface showed no significant differences in all the genotypes (Trived, 2014). In the present study, it was observed that the mean stomatal intex per square millimeter was more in case of the tolerant genotype as compared to the susceptible genotype.

In common bean susceptible varieties Bayo Madero showed a significant increase in proline concentration in, higher than that obtained for the drought resistant cultivar. Although the information on proline accumulation under drought in common beans is scarce, some studies have suggested that proline accumulates in drought-susceptible cultivars as a symptom of stress and not necessarily as a consequence of osmotic adjustment (Sanchez *et al*., 2010; Andrade *et al.,* 1995). An increase in proline was related with a decrease in leaf water status in drought susceptible genotype (Maggio *et al.,* 2002). In our results shows that susceptible varities is higher than the tolerant varieties. Leaf harvest wax amount was not related to other parameter such as specific leaf area and stomatal index (Mamrutha et al., 2010). Mulberry accessions elevated from the leaf surface wax amount and crystal size and density exhibited reduced leaf post harvest, water loss and could, proved the foundation for selective breeding of improve cultivators. The present study, indicates that the susceptible varieties have higher proline content than the tolerant varieties where, increased proline is related with decreased RWC.

CONCLUSIONS

In conclusion, this paper indicates that the parameters determined are (RWC), seedling root length, leaf area, stomatal index, wax and proline. The results obtained from this experiment show us that the tolerant varieties have higher water holding capacity than the susceptible varieties. Generally, tolerant varieties are higher in root length, leaf area and stomatal index and wax. In such cases, the root length and the leaf area were higher in susceptible varieties. There is no significant of both abaxial and adaxial surface of stomatal index. It is clearly observed that the proline content is higher in susceptible varieties when compared to the tolerant varieties. However, more proline accumulations are related to decrease RWC. A significant correlation was observed by using the parameters. The tolerant genotype could take up more water than the susceptible genotype and it maintains a better growth. The improvement of drought tolerance and susceptible varieties for common bean is a major objective for many breeding programs in different regions.

CONFLICT OF INTERESTS

The author declare that there is no conflict of interest.

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