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Nutritional and antioxidant properties and their inter-relationship with pod characters in an under-exploited vegetable, Indian bean (*Lablab purpureus*)

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ABSTRACT

Indian bean [*Lablab purpureus* (L.) Sweet] is an underexploited nutritious legume vegetable found in tropical regions of Asia and Africa. The nutritional and anti-oxidant properties of 21 pole type Indian bean genotypes were analysed in edible pods in terms of protein, sugar, chlorophyll, carotenoids, phenol, and proline contents. The analyses revealed a significant genotypic variation in the level of protein (102-635.6 mg), sugar (0.188-1.11 mg), chlorophyll (0.121-0.716 mg), phenol (1.7-9.67 mg), proline (0.02-7.06 µg) and carotenoids (0.04-0.231 mg). Estimation of genetic variability parameters revealed that chlorophyll a and non-reducing sugar had high estimates of PCV than GCV, whereas, protein, phenol, chlorophyll b, carotenoid, reducing sugar and non-reducing sugar had moderately high PCV than GCV indicating that such variability could be exploited for successful identification of genotypes for the specific biochemical property. In general, heritability estimates were recorded to be high for all the characters studied except chlorophyll a and reducing sugar. High heritability coupled with high genetic advance as percentage of mean was observed for proline, non-reducing sugar, chlorophyll a, carotenoidd, protein and phenol. Since such traits are controlled by additive genes, more importance need to be given to these traits while selecting the breeding lines rich in nutritional qualities.

Key words: Antioxidants, GCV, Hyacinth bean, Nutritional traits and PCV

Indian bean [*Lablab purpureus* (L.) Sweet] (common names, hyacinth bean and field bean) is an underexploited legume vegetable found in tropical regions of Asia and Africa (Rai *et al.* 2003, Rai *et al.* 2008). It is considered to be indigenous to India (Deka and Sarkar 1990). The green tender pods of Indian bean are used as vegetable. In addition, it is valued as forage and green manure (Pengelly and Maass 2001, Maass 2006). The green pods of Indian bean are good sources of protein, antioxidant, minerals and vitamins (Bradley 1999). The seeds of Indian bean are reported to be diuretic, anthelmintic, anti-spasmodic, aphrodisiac, digestive, carminative, febrifuge and stomachic (Chopra *et al*. 1986, Kirtikar and Basu 1995).

The nutritional value of legumes depends primarily on their biochemical and presence/absence of secondary metabolites and anti-nutritional factors. Carotenoids are thought to be responsible for preventing human diseases including cardiovascular diseases (Johnsen *et al.* 1995) cancer and other chronic diseases. Proline accumulates in many plant species under a broad range of stress conditions such as water shortage, salinity, extreme temperature and high light intensity (Delauney and Verna 1993, Hare *et al*. 1999, Mansour 2000, Claussen 2005). It is considered to be a compatible solute and protects folded protein structures against denaturation, stabilities cell membranes by interacting with phospholipids, function as hydroxyl radical scavenger, or serves as an energy and nitrogen source (Dlauney and Verna 1993, Hare *et al.* 1999). In potato, proline plays a major role in osmotic adjustments (Bussis 1998), while in tomato it accounts for only a small fraction of the total concentration of osmotically active solutes (Alfocea *et al*. 1993). Though extensive information is available on the nutritive values and their inter-relationship of many common legume vegetables like common bean (Tryphone and Msolla 2010) and faba bean (Kaul and Vaid 2003), but information on Indian bean is limited. Hence, the present study was aimed to analyse nutritional/anti-nutritional factors and their inter-relationship with edible pods properties of Indian bean with view to popularise Indian bean as nutrition-rich vegetable.

MATERIALS AND METHODS

A total of 21 diverse genotypes collected from different regions of India were grown at research farm of Indian Institute of Vegetable Research, Varanasi (Table 1). Field

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experiments were conducted in randomized block design with three replications during July to March of 2011-2012. The experimental site was located between 82.52ºE longitude and 25.10ºN latitude at an elevation of 128.93 m from mean sea level, and received an annual rainfall of ~1115 mm. The soil of experimental field was sandy loam, non-saline and neutral (pH 7.2) in reaction. Seeds were sown at row-to-row spacing of 3.0 m and plant-to-plant spacing of 1.5 m. Plants were trained on a bower system. In addition to 300 q/ha well-rotten farmyard manure, NPK was applied in the ratio of 60:60:60 kg/ha in form of urea (176 kg/ha), single superphosphate (390 kg/ha), and muriate of potash (90 kg/ha) for a good crop growth, and all the recommended in cultural operations were performed to raise a good crop.

For estimation of nutritional (protein, chlorophyll and sugar) and antioxidant (carotenoid, phenol and proline) factors, from each genotype, five random plant were chosen, and from each plant 10 marketable stage pods (25 d after anthesis) were collected; these 50 pods comprised one replication. These 50 pods were chopped into small pieces and used as per the requirement of biochemical estimation.

Total protein was estimated as per Lowry method (Lowry *et al*. 1951). For this purpose, one gram sample was crushed in 10 ml of (0.1M) Tris buffer and centrifuged. To the supernatant, 10% TCA was added and re-centrifuged, finally the pellet was dried and dissolved in 0.1 N NaOH, and absorbance was recorded at 650 nm using known concentrations of BSA. The concentrations of protein in the test samples were calculated from the calibration plot and expressed as mg protein per gram of sample.

The total sugar was measured by estimation of reducing and non-reducing sugar using the dinitrosalicylic acid (Miller 1972). One gram sample was extracted with 80% ethanol and centrifuged at 10000 xg for 20 min and the supernatant was saved. Supernatant was evaporated to remove ethanol and then the volume was made up to 10 ml using distilled water. Further, to 0.5 ml sample, 3.0 ml DNS (dinitrosalicylic acid) was added, and after heating for 5 min, 1.0 ml roschel salt was added, and finally, the absorbance of solution was recorded at 520 nm. In case of non-reducing sugar, the method of Malhotra and Sarkar (1979) was followed, where all the procedure was same as for reducing sugar, except the samples were neutralised with 1 ml 1N NaOH. Total sugar was expressed as g/100 g of fresh weight.

The chlorophyll and carotenoid was extracted in 80% acetone as per the protocol of Porra *et al*. (1983). The absorption of the extracts was recorded at 663 nm and 645 nm for chlorophyll and 480 nm and 510 nm for carotenoid, and the concentrations of chlorophyll a (Chl-a) and chlorophyll b (Chl-b) was calculated using the following equations (Arnon 1949):

Chlorophyll-a = 12.72A663-2.59A645

Chlorophyll-b = 22.9A645 -4.67A663

Total Chlorophyll = Chlorophyll-a + Chlorophyll-b

The total phenolic contents were determined according to the method of Imeh and Khokhar (2002). For this, 1.5 ml of extract was taken and 0.1 ml of Folin coicalteau reagent and 0.3 ml Na_2CO_3 (20%) were added sequentially. The test solution was warmed for 30 min at 40ºC; later cooled to room temperature and absorbance was recorded at 650 nm. The concentrations of phenols in samples were calculated from the catechol calibration plot and expressed as mg catechol equivalent of phenol/g of sample.

Fresh green seed samples were used for extraction and colorimetric determination of phenol (Bates *et al*. 1973). The extraction buffer contained acidic ninhydrin reagent (2.5 g/100 ml of a solution containing glacial acetic acid, distilled water and ortho-phosphoric acid (85%) in the ratio of 6:3:1). Samples of 1 g were ground in 10 ml of a 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was filtered, and the clear filtrate was then used for assay. Glacial acetic acid and ninhydrin reagent (1 ml each) were added to 1 ml of the filtrate. The reaction mixture was boiled in a water bath for 1 h, and the reaction was terminated by transferring to ambient temperature for 5 min, and finally, 1 ml of toluene was added, and absorbance was recorded at 546 nm. The proline concentration was determined from a standard curve and expressed on a fresh weight basis as µmol proline/g.

For each trait value, mean±standard error and the least significant difference (LSD) was calculated at P >0.05. Analysis of variance (ANOVA) was done as per the method of Panse and Sukhatme (1989). Various parameters of phenotypic and genotypic variability, heritability for nutritional (protein, total sugars and chlorophylls) and antioxidants (Crotenoids, phenol and proline) were estimated as per the method of Johnson *et al*. (1955) and their inter-relationship were computed as per the method of Dewey and Lu (1959) and Rao *et al.* (1981). The genetic advance as calculated as per the formulae GA= kvVp H^2 (Johnson *et al.* (1955). Where, GA = the expected genetic advance, k = selection intensity (2.06), = vVp the phenotypic standard deviation. GA expressed as percentage of mean was calculated with the following formula:

$$\text{GA expressed as percentage of mean} = \frac{\text{GA}}{\text{x}} \times 100$$

RESULTS AND DISCUSSION

Variability

A significant and wide variation was recorded for nutritional factors and anti-oxidants in 21 genotypes of Indian bean (Table 1). The biochemical estimations revealed the maximum total protein concentration was recorded in the genotype VRSEM-3 (635.6 mg/g) followed by the genotype VRSEM-894 (582.6 mg/g), it was the lowest in genotype VRSEM-201. The total sugar was estimated to be the highest in genotype VRSEM- 752 (1.11 g/100 g), followed by VRSEM-67 (1.056 g/100 g); the lowest total sugar was exhibited by the genotype VRSEM-501 (0.188 g/100 g). In the case of winged bean, Ningombam *et al.* (2012) reported total sugar from 0.415 g to 0.488g. Whereas, in the case of *L. perpureus*, it ranged between 0.4 g and

1.93 g (Hooda *et al*. 1999).

The genotype, VRSEM-6 contained the highest total chlorophyll (0.716 mg/g) followed by VRSEM-894 (0.493 mg/g); the lowest was exhibited by the genotype VRSEM-97 (0.082 mg/g). These results are in agreement with the findings of (Santos *et al*. 2009), where chlorophyll was recorded in the range of 0.360-0.711 mg in common bean and they also suggested that chlorophyll concentration decreases with an increase in pod length. The amount of Chlorophyll a (0.181) was about three-folds higher than chlorophyll b (0.082). Gross (1991) reported that in higher plants, chlorophyll a was the major pigment and chlorophyll b was an accessory pigment, and a/b ratio was generally around 3:1. In the case of *Oryza sativa*, Lin (2002) reported that the chlorophyll a: chlorophyll b ratio was 3:2. Carter and Knapp (2001) reported that green tissues of higher plants contain 67-78% more chlorophyll and formed much more rapidly compared to chlorophyll b.

Among the antioxidants, the total carotenoid ranged from 0.044 mg/g (VRSEM-97) to 0.231 mg/g (VRSEM-6). The highest phenol content was recorded in the genotype VRSEM-764 (9.67 mg catechol/g) followed by VRSEM-186 (4.75 mg catechol/g), in contrast, VRSEM-749 showed the lowest (1.7 mg catechol/g) phenol content. Among the 21 genotype, a wide variation was recorded for proline content, it was recorded to be the highest (7.01 µg/g) in VRSEM-501, and the lowest (0.02 µg/g) in VRSEM-860. The genotypes recording the highest content of protein (VRSEM-3), chlorophyll and carotenoids (VRSEM-6) were a collection from Tripura, whereas, some of the genotypes collected from Uttar Pradesh showed maximum amount of sugar (VRSEM-752), phenol (VRSEM-764). These observations indicate that Tripura and Uttar Pradesh states need to be explored to collect new genotypes or germplasm, which could show a higher nutritional content. Hosfield *et al*. (1984) suggested environ specific adaptation of varieties for nutritional traits. In order to test such hypothesis genotype to environmental interactions need to be analysed.

The protein content in the genotypes varied between 5.1% (VRSEM-201) and 31.1% (VRSEM-3), with majority of genotypes in the higher range. Such results are in agreement to reports of Baudoin and Maquet (1999) where the protein content in 14 genotypes of *P. vulgaris* ranged was recorded to be 19.1-29.7%. Similarly, in case of wild bean (*Phaseolus vulgaris*) seeds, a higher (18.0-33.0%) range was reported by Guzman Maldonado (2000). With a higher content of lysine- rich protein (15.0-30.0%) having 55% digestibility hyacinth bean could be a very good nutritional supplement for majority of malnourished children, pregnant women and old people.

Phenols are produced in response to certain pathogen and are considered to be essential for the growth and reproduction of plants. The phenol content in the genotypes used in the present study was in conformity to the findings of Sosulski and Dabrrowski (1990) where it was recorded to be 6.9 mg/g and in dry bean it was 19.1 mg/g. The level of phenolic compounds is generally influenced by genotype, agronomic practices, and maturity at harvest, post-harvest storage and climatic conditions (Dixon and Paiva 1995, Ninfali and Bacchiocca 2003, Hakkinen and Torronen 2000).

The distribution of carotenoids in plants appears to be shaped by the changes in their physiological, biochemical and genetic factors (Goldman *et al*. 1999, Grusak *et al*. 1999). In present study, carotenoid content ranged between 0.04 mg/g and 0.231 mg/g. Siong (1995) recorded significant differences in carotenoids content in legumes vegetables, i.e. kidney bean (0.017 mg/g), cowpea (0.051 mg/g), and soybean (0.229 mg/g) and concluded that carotenoid content was much lower compared to other vegetables and fruits. Similarly, leafy vegetables generally contain a much higher (~10 folds) carotenoids (Tee and Lim 1991b). Dietary intake of carotenoids is reported to reduce the risk of lung cancer in human (LeMarchand *et al*. 1993) and eye diseases (Johnson *et al*. 1995).

Proline is a compatible solute which accumulates in many plant species under stress conditions such as water-deficit, salinity, high temperatures and light intensity (Mansour 2000, Delauney and Verna 1993, Hare *et al*. 1999). It protects folded protein structures against denaturation, stabilises cell membranes by interacting with phospholipids, acts as a hydroxyl radical scavenger, or break down in to amino acids and nitrogen source to provide energy. Under normal conditions, the proline concentration varied between 0.069-7.06 µg/g. However, in previous studies, under stress conditions, a much higher level of proline accumulation has been reported, for example, Raggio *et al*. (2007) reported 167.4-485.9 mg proline in common bean plants.

Heritability and genetic advancement

A wide variability in the genetic stocks provides a better chance of producing desired genotype of a crop plant. One of the main objectives of germplasm conservation is to collect and preserve the genetic pool in a given crop species so that it could be exploited in future breeding programmes. In present study, low, moderate as well as high PCV and GCV values were obtained for different traits (Table 2). The higher PCV estimates compared to GCV for the traits like protein, phenol, chlorophyll a, chlorophyll b, reducing sugar, non-reducing sugar and proline indicated the strong influence of environment on the expression of these traits. Therefore, selection on the basis of phenotype alone cannot be effective for the improvement of these traits. A number of reports are available on variability, heritability and genetic advance for morphological traits in Indian bean (Rai and Yadav 2005, Rai *et al*. 2008). But a few information is available for variability, heritability and genetic advance for nutritional and antioxidants traits in Indian bean.

The extent of heritability acts as effective parameters in expressing the reliability of phenotypic value, thus a high heritability helps in effective selection for a particular trait. Present investigation revealed that heritability ranged from 47.6 to 99.2% (Table 2). Chlorophyll a present in edible

pods recorded the highest heritability and chlorophyll b recorded the lowest heritability. In the case of rice, high heritability for nutrition and anti-nutritional qualities has previously been reported by Babu *et al*. (2012) for amylose (93%), zinc (85.8%), iron (51.5%) and protein contents (31.7%). Such high heritability values indicate that expression of characters under study is less influenced by environment. Plant breeders on such basis may make the selections safely on the basis of phenotypic expression of characters in the individual plant by adopting simple selection methods.

In variability studies, genetic advance is useful indicator of the progress that can be expected as result of performing selection on the relevant population. Heritability in correlation with genetic advance would give a more reliable index of selection value (Johnson *et al*. 1955). The genetic advance was recorded to be high for protein and the lowest for chlorophyll b and reducing sugar (Table 2). The genetic advance as per cent of mean was the highest in the case of phenol, while it was the lowest for chlorophyll b (1.81-15.59). Genetic advance as per cent mean for nutritional (protein 14.65) and anti-nutritional (amylose content 10.25) quality traits was also reported by Basu *et al*. (1999) in Indian bean which is correlated well with our findings in hyacinth bean. The information on genetic variation, heritability and genetic advance helps to predict the genetic gain that could be obtained in later generations, if selection is made for improving the particular trait under study.

Path analysis

Path coefficient is the standardized partial regression coefficients that offer the measure of direct and indirect influence on one variable on the other (Table 3). Path analysis revealed that phenol, chlorophyll a and proline had positive, while protein, chlorophyll b, and reducing sugar had negative direct effect on non-reducing sugar. The maximum positive direct effect was exhibited by chlorophyll a on chlorophyll b (0.926) and the minimum positive direct effect was exhibited by carotenoid on proline (0.0017). The maximum negative indirect effect was shown by non-reducing sugar on protein (-0.321) while the minimum was exhibited by protein on chlorophyll a (–0.03). Protein exhibited negative indirect effects on phenol (–0.06), chlorophyll a (–0.03), reducing sugar (–0.09) and non-reducing sugar (–0.321), whereas, positive indirect effects was recorded on carotenoid (0.031), chlorophyll b (0.14) and proline (0.02). Phenol showed positive indirect effects on chlorophyll a (0.56), chlorophyll b (0.19), carotenoid (0.223), reducing sugar (0.41) proline (0.17) and non-reducing sugar (0.23) and a negative indirect effect on protein (-0.06). Chlorophyll a exhibited positive indirect effect on phenol (0.56), chlorophyll b (0.926), carotenoid (0.908), proline (0.306), reducing sugar (0.31) and non-reducing sugar (0.05), whereas, negative indirect effect only on protein (-0.03). Chlorophyll b has exhibited positive indirect effect on protein (0.14), phenol (0.19), chlorophyll a (0.926), carotenoid (0.89), and reducing sugar (0.10), whereas, negative indirect effect was shown on non-reducing sugar. Carotenoid showed positive indirect effect on protein, phenol, chlorophyll a, chlorophyll b and proline, whereas, negative indirect effect on reducing and non-reducing sugar. Proline showed positive indirect effect with all the traits. Reducing sugar exhibited positive indirect effect on phenol, chlorophyll a, and b, carotenoid and proline, whereas negative indirect on protein and non-reducing sugar. Since the information on path analysis in Indian bean for nutritional and antioxidant factors is not available, nevertheless, it can be concluded that negative correlation observed between some of the factors may result in reduction of rate of improvement compared to what could have been attained if the correlation was positive.

Correlation

Phenotypic and genotypic correlations of eight biochemical traits were analysed to decipher their inter-relationship (Table 4). Such analysis suggests as which trait may be useful in a selection program (Jhonson *et al*. 1955). Chlorophyll a showed the maximum positive and significant association with chlorophyll b (0.925) and carotenoid (0.908) at both genotypic and phenotypic levels (Table 4). Similarly, Chlorophyll b also showed a positive and significant association with carotenoid (0.897) at both genotypic and phenotypic levels. Proline showed a positive and significant association with reducing sugar (0.606) both at phenotypic and genotypic levels. Phenol showed a positive and significant correlation with chlorophyll a (0.560) and reducing sugar (0.426). Chlorophyll a showed positive and significant correlations with proline (0.306) and reducing sugar (0.318). Protein showed positive correlations with chlorophyll b (0.141), carotenoid (0.031) and proline (0.024). Phenol was also found to be positively associated with chlorophyll b (0.195), carotenoid (0.223), proline (0.178) and non-reducing sugar (0.238). Chlorophyll a showed positive association with non-reducing sugar (0.055). Chlorophyll b also showed positive correlation with proline (0.136) and reducing sugar (0.105). Proline showed positive association with non-reducing sugar (0.154). Chlorophyll a had the maximum significant value for chlorophyll b (0.925) followed by carotenoid (0.897) and reducing sugar (0.318). In present study, for most of the nutritional and anti-nutritional qualities the genotypic correlation coefficient was recorded to be higher than phenotypic correlation coefficient. Similar results were also reported in the case of cowpea (Saha *et al*. 2009).

A wide variability in the genetic stocks provides a better chance of producing desired genotype of a crop plant. One of the main objectives of germplasm conservation is to collect and preserve the genetic pool in a given crop species so that it could be exploited in future breeding programmes. In present study, low, moderate as well as high PCV and GCV values were obtained for different traits. The higher PCV estimates compared to GCV for the traits like protein, phenol, chlorophyll a, chlorophyll b, reducing sugar, non-reducing sugar and proline indicated

the strong influence of environment on the expression of these traits. High heritability coupled with high genetic advance as percentage of mean was observed for proline, non-reducing sugar, chlorophyll a, carotenoid, protein and phenol. Therefore, the present study indicated that there was strong influence of environment on the expression nutritional and anti-nutritional traits like protein, phenol, chlorophyll a, chlorophyll b, reducing sugar, non-reducing sugar and proline as evidenced by higher PCV estimates compared to GCV. Therefore, selection on the basis of phenotype alone cannot be effective for the improvement of these traits.

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