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Agrobotanical Attributes, Nitrogen-Fixation, Enzyme Activities and Nutraceuticals of Hyacinth Bean (*Lablab purpureus* L.): A Bio-Functional Medicinal Legume

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Abstract: Hyacinth bean (*Lablab purpureus* L.) accessions of different origins received from USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, GA, USA were evaluated for agrobotanical attributes, enzyme activities, nutraceuticals and quality in calcium deficient soil of Aligarh, Western Uttar Pradesh, India. Fresh and dry weights per plant, leaf-area, number and dry weight of nodules per plant, net photosynthetic rate, stomatal conductance and transpiration rate, total chlorophyll and carotenoid content, activities of nitrate reductase and carbonic anhydrase, leaf - N, P, K and Ca contents and nodule-nitrogen and leghaemoglobin contents, respectively were analyzed at 60, 90 and 120 day after sowing. Photosynthesis was measured only at 90 DAS. Yield attributes including pod number per plant, seed number per pod, 100-seed weight and seed-yield per plant were recorded at harvest (150 DAS). Protein and carbohydrate content as well as tyrosinase activity in hyacinth bean seeds were also determined. Among the five accessions, EC-497619 (A_4) showed superior performance over the rest of the accessions. Accession A_4 showed the highest values for growth, yield, physiological, biochemical and quality attributes in comparison to the other accessions. Net photosynthetic rate, stomatal conductance and transpiration rate were found maximum in the A_4 accession. Chlorophyll and carotenoid content were also reported higher in accession A_4. Accession A_4 showed higher nitrate reductase and carbonic anhydrase activities than the other accessions. Nodule-nitrogen and leghaemoglobin content ranged from 5.267-5.314% and 0.110-0.130 mM, respectively. Mineral profiles, viz., nitrogen, phosphorus, potassium and calcium content varied from 3.610-3.643, 0.338-0.356, 3.020-3.124 and 1.764-1.804%, respectively. Seed protein of all accessions varied from 24.70-25.06%. Carbohydrate content ranged from 50.83-53.16% across all accessions tested. Accession A_4 produced the highest tyrosinase activity in the seeds.

Key words: *Lablab purpureus* L., nitrate reductase activity, carbonic anhydrase activity, tyrosinase activity, nutraceuticals

INTRODUCTION

The vegetarian populations in India consume large amounts of legumes, particularly, vegetable beans in their diet. The beans are naturally rich in carbohydrates, proteins, fat and fibers as well as minerals including calcium, phosphorus and iron. Furthermore, several legumes have tremendous potential as nutraceuticals because of their healing properties (Morris, 1999, 2003). Hyacinth bean belongs to the family Fabaceae and is grown in the tropical and sub-tropical India. Hyacinth bean

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(*Lablab purpureus* L.) has great potential as medicinal legume. Among the legumes, hyacinth bean constitutes an important source of therapeutic agents used in the modern as well as traditional systems of medicine (Morris, 1996, 1999, 2003). It carries tremendous healing potential. In fact, it is considered a multipurpose crop since it is used for food, forage, soil improvement, soil protection and weed control (Shivashankar and Kulkarni, 1989; Karachi, 1998; Morris, 1997, 2003; Pengelly and Maass, 2001; Maass, 2006). The young pods and tender beans are used as vegetables in India and tropical and warm temperate Asia. It is also been known for its use as a green manure and produces edible young pods, dried seeds, leaves and flowers (Morris, 1997, 2003). The seeds are used as a laxative, diuretic, anthelmintic, antispasmodic, aphrodisiac, anaphrodisiac, digestive, carminative, febrifuge and stomachic (Chopra *et al*., 1986; Kirtikar and Basu, 1995). Hyacinth beans contain fiber which is known to prevent cancer, diabetes, heart disease, obesity and is used as a laxative (Beckstrom-Sternberg and Duke, 1994). Hyacinth bean contains the potential breast cancer fighting a flavonoid known as kievitone (Hoffman, 1995). The flavonoid, genistein found in hyacinth bean may play a role in the prevention of cancer (Kobayashi *et al*., 2002) and as a chemotherapeutic and/or chemopreventive agent for head and neck cancer (Alhasan *et al*., 2001). Tryrosinase (polyphenol oxidase) is present in plant tissue and is important in fruit and vegetable processing as well as storage of processed foods. Prevention of browning of foods, enzymatic or nonenzymatic, has long been a concern of food scientists (Matheis, 1987; Sanchez-Ferrer *et al*., 1995; Paul and Gowda, 2000). Hyacinth bean contains tyrosinase, which has potential for the treatment of hypertension in humans (Beckstrom-Sternberg and Duke, 1994). However, it is not being used to its full potential.

Keeping in mind the importance of this medicinal legume as a multipurpose crop, the aim of the present study is to investigate the performance of five hyacinth bean accessions of different origin, for various agrobotanical attributes, physiological, biochemical and quality attributes under the agro-climatic conditions of Aligarh, Western Uttar Pradesh in calcium deficient soil.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Healthy seeds of hyacinth bean (*Lablab purpureus* L.) accessions, namely EC-497617 (Australia), EC-497616 (China), EC-497615 (Egypt), EC-497619 (Iran) and EC-497618 (Kenya) were received from the USDA, ARS, Plant Genetic Resources Conservation Unit, Griffin, GA, USA and denoted as A_1, A_2, A_3, A_4 and A_5, respectively. Healthy seeds of uniform size were selected and their viability was tested using 1% tetrazolium salt. The seeds were surface sterilized with 95% ethyl alcohol for five minutes and then washed thoroughly with distilled water.

Healthy and viable *Rhizobium* culture (*Rhizobium* sp.), compatible for hyacinth bean, was obtained from Culture Laboratory, Government Agriculture Farm, Quarsi, Aligarh. The *Rhizobium* culture was prepared according to Subba Rao (1972). Two hundred grams of colorless Gum Arabic (coating material) and 50 g of sugar were dissolved in 500 mL warm water. After cooling, 100 g of *Rhizobium* culture was mixed with the solution. The required amount of seeds were mixed vigorously with the inoculum until they were evenly coated by the inoculum mixture. The inoculated seeds were placed in a clean tray and dried in shade prior to sowing. Then seeds were sown directly at a depth of 2 cm in soil into earthen pots containing a homogenous mixture of soil and farmyard manure (5:1). Initially five plants were maintained in each pot, but later were reduced to one healthy plant. The soil was maintained at proper moisture to ensure better seed germination. Physico-chemical characteristics of the soil were: texture-sandy loam, pH (1:2), 7.2, E.C. (1:2), 0.46 m mhos cm^{-1}, available N, P and K 98.39, 6.78 and 142.9 mg kg^{-1} soil, respectively and calcium carbonate 0.12%. The soil samples were analyzed at the Government Soil Testing Laboratory, Quarsi Farm, Aligarh.

Prior to seed sowing, a uniform basal dose of fertilizers (10 mg P and 120 mg Ca kg^{-1} soil) was applied. The source of phosphorus and calcium were potassium dihdrogen orthophosphate and calcium chloride. However, being a leguminous crop, hyacinth bean was not supplied with nitrogen. The requirement of nitrogen is fulfilled by the crop by virtue of biological nitrogen fixation since *Rhizobium* was applied to the seeds. An out door pot experiment was conducted during 2002-2003 using a simple randomized complete block design in the net-house at the Botany Department, AMU, Aligarh (27° 52' N latitude, 78° 51' E longitude and 187.45 m altitude). The crop was sown on 20th September, 2002 and harvested on 20th February, 2003. Each accession was replicated three times. The pots were watered thoroughly and plants were grown in natural condition.

Measurement of Growth and Yield Attributes

At 60 (vegetative stage), 90 (flowering stage) and 120 (pod-filling stage) days after sowing, three plants of each accession were uprooted carefully and washed with running tap water to remove foreign particles. Fresh plant weight was recorded. Leaf area was measured by outlining leaves of sampled plants on graph paper and dry weight of these leaves was recorded. Leaf-area per plant was determined using leaf dry weight per plant and dry weight of those leaves for which the area was estimated (Watson, 1958). The root nodules of each plant were washed under tap water and counted. The plants were then dried at 80°C for 24 h and the dry weights of nodules and plants were recorded.

At harvest (150 DAS), six plants from each accession were uprooted randomly and were used for computing yield attributes including pod number per plant, seed number per pod, 100-seed weight and seed-yield per plant. Pods were threshed and cleaned. The seeds cleaned and counted. Seeds were sun dried for recording a more accurate 100-seed weight. Seed-yield was calculated accordingly.

The fresh leaves of each accession were used for analysis of various physiological and biochemical attributes except leaf-N, P, K and Ca contents.

Net Photosynthetic Rate, Stomatal Conductance and Transpiration Rate

Net photosynthetic rate, stomatal conductance and transpiration rate were measured on cloud-less clear days at 1100 h from fully expanded hyacinth bean leaves using a IRGA (Infra Red Gas Analyzer, LICOR 6200 Portable Photosynthesis System, Lincoln, Nebraska, USA). Before recording the measurement, the IRGA was calibrated and zero was adjusted approximately every 30 min during the measurement period. The atmospheric conditions during measurement were Photosynthetically Active Radiation (PAR) 1016±6 1 µmol/m^2/sec, relative humidity 60±3%, atmospheric temperature 22±1°C and atmospheric CO_2 360 µmol mol^{-1}. The ratio of atmospheric CO_2 to intercellular CO_2 concentration was constant. Leaves of each accession were enclosed in a 1 L gas exchange chamber for 60 sec. All attributes were measured three times for each accession. Photosynthesis was measured only at 90 days after sowing.

Total Chlorophyll and Carotenoid Content

Total chlorophyll and carotenoid content in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). The fresh tissues from interveinal leaf area were ground in a mortar and pestle containing 80% acetone. The Optical Density (OD) of the solution was read at 662 and 645 nm (chlorophyll a and b) and 470 nm (carotenoids) using a spectrophotometer (Spectronic 20D, Milton Roy, USA). Photosynthetic pigments were expressed as mg g^{-1} FW.

Nitrate Reductase Activity (NRA)

The enzyme activity was estimated by the intact tissue method developed by Jaworski (1971). Two hundred milligrams of fresh chopped hyacinth bean leaves were weighed and transferred to a

plastic vial. Each vial contained 2.5 mL phosphate buffer (pH 7.5) and 0.5 mL potassium nitrate solution followed by the addition of 5% isopropanol. After incubation, 1% sulphanilamide and 0.02% N-(1-Naphthyl) ethylenediamine dihydrochloride (NED-HCL) was added. The OD of colour was read at 540 nm using a spectrophotometer. Nitrate reductase activity was expressed as nM NO_2^- g^{-1} FW h^{-1}.

Carbonic Anhydrase Activity

The Carbonic Anhydrase (CA) activity in fresh leaves was analyzed using the method described by Dwivedi and Randhawa (1974). Two hundred milligram of fresh leaf pieces were weighed and transferred to petri plates. The leaf pieces were dipped in 10 mL of 0.2 M cystein hydrochloride for 20 min at 4°C. Four mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022% bromothymol blue was added to the homogenate. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. Carbonic anhydrase activity was expressed as μM CO_2 kg^{-1} leaf FW sec^{-1}.

Nutrient Analysis

Leaf samples of each accession were digested according to the method used by Lindner (1944) for the estimation of leaf -N, P, K and Ca contents.

Leaf-Nitrogen Content

Leaf-nitrogen content was estimated using the method of Lindner (1944) as well. A 10 mL aliquot (peroxide-digested material) was taken in a 50 mL volumetric flask. To this, 2 mL of 2.5 N sodium hydroxide and 1 mL of 10% sodium silicate solutions were added to neutralize the excess of acid and to prevent turbidity, respectively. In a 10 mL graduated test tube, 5 mL aliquot of this solution was taken and 0.5 mL Nessler's reagent was added. The contents of the test tubes were allowed to stand for 5 min for maximum colour development. The OD of the solution was read at 525 nm, using a spectrophotometer. The reading of each sample was compared with the standard calibration curve of ammonium sulphate to estimate the percent nitrogen content.

Leaf-Phosphorus Content

The method of Fiske and Subba Row (1925) was used to estimate the leaf-phosphorus content in the digested material. The same aliquot was used to determine the leaf-P content. A 5 mL aliquot was taken in a 10 mL graduated test tube where 1 mL molybdic acid (2.5%) was added carefully, followed by addition of 0.4 mL 1-amino-2-naphthol-4-sulphonic acid. When the colour of tube turned blue, the volume was made up to 10 mL with the addition of double distilled water. The solution was shaken for 5 min. The OD of the solution was read at 620 nm using a spectrophotometer.

Leaf-Potassium and Calcium Content

Potassium and calcium contents were analyzed using flame-photometrics. In the flame-photometer, the solution (peroxide-digested material) is discharged through an atomizer in the form of a fine mist into a chamber, where it is drawn into a flame. Combustion of the elements produces light of a particular wavelength (λ_{max} for K = 767 nm (violet)). The light produced was conducted through the appropriate filters to impinge upon a photoelectric cell that activates a galvanometer. Both leaf potassium and calcium content in the same aliquot were estimated and recorded with the help of emission spectra using specific filters in a flame-photometer. Leaf-N, P, K and Ca content were expressed in percent on the dry weight basis.

Nodule-Nitrogen and Leghaemoglobin Content

Nodule-nitrogen content was also estimated by the method of Lindner (1944). Leghaemoglobin (Lb) content in fresh nodules was determined as described by Sadasivam and Manickam (2008). The solution's OD was recorded at 556 and 539 nm. The Lb content was calculated using the following formula:

$$\text{Lb concentration (mM)} = \frac{\text{OD } 556 - \text{OD } 539 \times 2\text{D}}{23.4}$$

where, OD 556 and 539 represent absorbance (OD). Values recorded at 556, 539 nm, respectively and D is the initial dilution.

Seed-Protein Content

The seed protein content was estimated using the method of Lowry *et al.* (1951). Hyacinth bean seed was ground to a powder using a mortar and pestle. The seed powder was transferred to a mortar where 5% cold trichloroacetic acid (TCA) was present. Extracted protein was measured at 660 nm using a spectrophotometer. The reading was compared with a calibration curve obtained by using known dilution of standard egg albumin solution and the percent seed protein content was calculated on a dry weight basis.

Total Carbohydrate Content

Total carbohydrate content in seeds was analyzed as described by Sadasivam and Manickam (2008). One hundred milligrams of hyacinth bean powder was poured into a tube containing boiling sulphuric acid and centrifuged at 4000 rpm. Four milliliter of anthrone reagent was added and the resulted dark green colour was recorded at 630 nm. The reading was compared with the calibration curve obtained using a known dilution of a standard of glucose and the percent carbohydrate content was calculated on a dry weight basis.

Determination of Tyrosinase (Polyphenol Oxidase) Activity

The tyrosinase enzyme was extracted according to Paul and Gowda (2000) and assayed spectrophotometrically, using the procedure of Cosetang and Lee (1987). The enzyme assay mixture contained 0.9 mL of 0.05 M sodium acetate buffer (pH 4.5), 0.1 mL of substrate (L-3, 4-dihydroxy phenylalanine) (L-DOPA) and 10-100 μg of the enzyme. The optical density of coloured solution developed due to formation of the compound dopachrome was read at 480 nm. One unit of the enzyme activity corresponded to an amount of enzyme that caused an increase in the absorbance of 0.001 min^{-1} at 25°C. The reference cuvette contained all the ingredients except the enzyme in a final volume of 1 mL. The activity of tyrosinase was expressed as U mg^{-1} protein.

Statistical Analysis

The data were analyzed by one-way ANOVA. Mean values were analyzed at the 0.05 level of probability according to Gomez and Gomez (1984).

RESULTS

Growth and Yield Attributes

The mean data and Least Significant Differences (LSD) ($p \leq 0.05$) of agrobotanical attributes from five hyacinth bean accessions are presented in Table 1 and 2. Accession A_4 followed by A_1 had the greatest fresh and dry weights per plant at 60, 90 and 120 DAS. Accession A_5 had the lowest fresh and dry weights at 60, 90 and 120 DAS. Both accession A_4 and A_1 produced the greatest leaf-area

Table 1: Growth attributes of five accessions of hyacinth bean (*Lablab purpureus* L.) studied at 60, 90 and 120 DAS

		Hyacinth bean accessions					
Attributes	DAS	A_1	A_2	A_3	A_4	A_5	LSD at 5%
Fresh weight plant^{-1} (g)	60	23.530b	22.460c	22.140c	25.300a	15.640d	1.050
	90	53.160b	50.760c	52.240c	55.710a	32.890d	2.380
	120	75.200a	72.300b	73.120b	76.140a	43.900c	2.030
Dry weight plant^{-1} (g)	60	4.500b	4.200c	4.260c	5.120a	4.000d	0.210
	90	8.980b	8.460c	8.710bc	10.810a	7.900d	0.480
	120	12.730b	11.240c	11.660c	14.530a	10.460d	0.745
Leaf-area plant^{-1} (cm^2)	60	1420.900a	1306.700c	1314.000b	1426.400a	1298.500d	7.230
	90	1780.200a	1590.400c	1762.300b	1786.500a	1583.000c	12.160
	120	1939.500b	1754.800d	1928.900c	1974.300a	1746.000d	10.300
No. of nodules plant^{-1}	60	28.000b	25.000c	27.300b	31.300a	20.000d	1.800
	90	46.000b	43.000c	45.000b	49.000a	41.000d	1.840
	120	31.700b	30.000b	31.300b	33.700a	28.000c	1.900
Dry weight of nodules	60	0.250b	0.218c	0.244b	0.263a	0.218c	0.016
plant^{-1} (g)	90	0.461b	0.380cd	0.394c	0.478a	0.364d	0.016
	120	0.276a	0.234c	0.256b	0.280a	0.224 c	0.019

Mean values of 3 replicates. Mean values within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$)

Table 2: Yield attributes of five accessions of hyacinth bean (*Lablab purpureus* L.) studied at 150 DAS

		Hyacinth bean accessions					
Attributes	DAS	A_1	A_2	A_3	A_4	A_5	LSD at 5%
No. of pods plant^{-1}	150	43.00b	40.00c	42.00b	45.20a	32.00d	1.95
No. of seeds pod^{-1}	150	4.30	4.00	4.00	4.50	4.00	NS
100-seed weight (g)	150	31.58a	29.89b	30.14b	33.24a	28.86c	0.96
Seed-yield plant^{-1} (g)	150	44.16ab	42.89bc	42.10c	45.60a	34.64d	1.40

NS: Not Significant: Mean values of 3 replicates. Mean values within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$)

among all accessions at 60, 90 and 120 DAS. Accession A_5 produced the least amount of leaf-area at all growth stages (Table 1). Nodule number and dry weight were highest in accession A_4 at 60, 90 and 120 DAS, while accession A_5 produced the lowest number of nodules and nodule dry weight at similar stages (Table 1).

Table 1 indicates that accession A_4 produced the highest number of pods per plant (45.2), 100-seed weight (4.5 g) and seed-yield (45.60 g) per plant, while accession A_5 produced lowest yield (34.64 g). However, all accessions produced similar values for seed number per pod and did not significantly differ from each other (Table 2).

Physiological and Biochemical Attributes

The net photosynthetic rate was maximized in accession A_4 followed by accession A_1. Accession A_5 had the lowest rate at 90 DAS (Fig. 1). It was observed that stomatal conductance and transpiration rate maximized in accession A_4, while accession A_1 was similar with that of A_4. Accession A_5 had the lowest transpiration rate and stomatal conductance (Fig. 1). Both accessions, A_4 and A_1 produced the highest chlorophyll content and were superior to the other accessions at 60, 90 and 120 DAS (Fig. 1). Both accessions A_5 and A_2, had similar chlorophyll content at 60, 90 and 120 DAS ($p \leq 0.05$). Maximum carotenoids were generated in the leaves of accessions A_4 and A_1 at 60 DAS followed by A_3, A_2 and A_5 at all three DAS (Fig. 1). Maximum total chlorophyll (1.889 mg g^{-1}) and carotenoid (0.568 mg g^{-1}) content were found in all accessions at 90 DAS. Accession A_4 had the maximum nitrate reductase activity and was similar to accessions A_1 and A_3. Accession A_5 produced the lowest NR activity (Fig. 1). It was observed that A_4 produced the highest carbonic anhydrase activity and accession A_5 produced the lowest at all three growth stages of the hyacinth bean plant (Fig. 1). Nodule-nitrogen content was maximum in accession A_4 (5.314, 4.439 and 3.435% at 60, 90 and

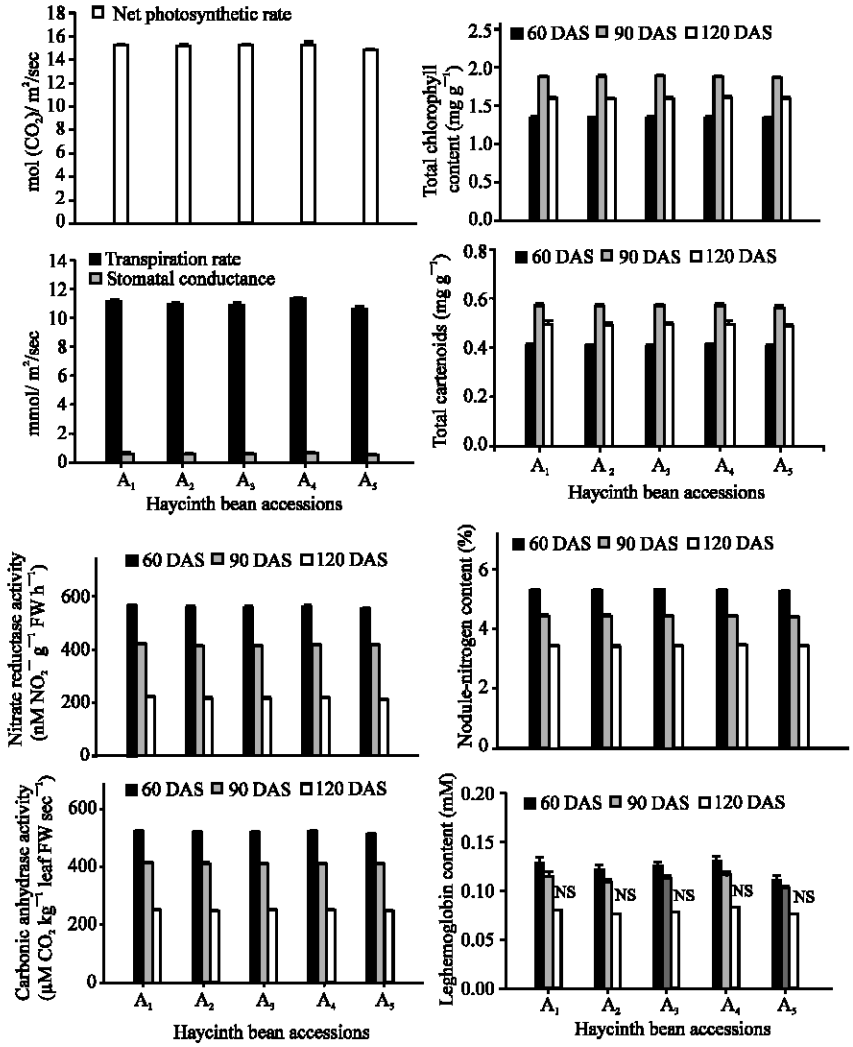

Fig. 1: Changes in net photosynthetic rate, stomatal conductance and transpiration rate (90 DAS), total chlorophyll and carotenoid content, nitrate reductase activity, carbonic anhydrase activity, nodule-nitrogen content and leghaemoglobin content of hyacinth bean (*Lablab purpureus* L.) accessions studied at 60, 90 and 120 DAS. Error bars (⊤) show LSD at 5% level

120 DAS). However, accessions A_1 (5.310, 4.434 and 3.430%) and A_4 (5.314, 4.439 and 3.435%) were not significantly different and were almost equal in nodule-nitrogen content at 60, 90 and 120 DAS (Fig. 1). Accession A_5 produced the lowest nodule nitrogen content at all three stages. In the present study, maximum nodule-nitrogen content was found at 60 DAS in all the accessions (Fig. 1).

Accessions A_4 and A_1 produced the highest concentration of leghaemoglobin content at 60 and 90 DAS while accession A_5 produced the least (Fig. 1). Nitrogen is the most abundant macro element and was highest in accession A_4 followed by accession A_1 at 60, 90 and 120 DAS. Accession A_5 produced the lowest amount of nitrogen at all growth stages (Fig. 2). Potassium content among

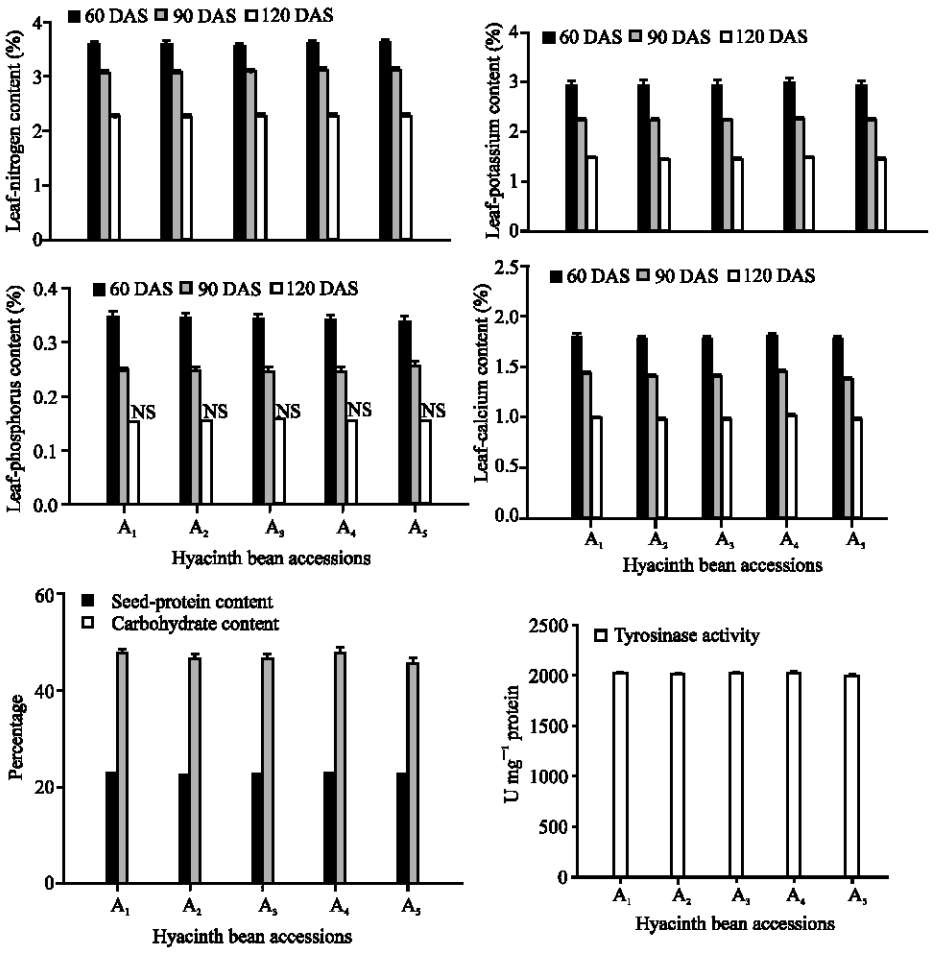

Fig. 2: Changes in leaf-nitrogen, phosphorus, potassium and calcium contents (60, 90 and 120 DAS), seed-protein content, carbohydrate content and tyrosinase activity of hyacinth bean (*Lablab purpureus* L.) accessions analyzed at 150 DAS. Error bars (⊤) show LSD at 5% level

hyacinth bean accessions occupied the second position followed by calcium and phosphorus concentrations at 60, 90 and 120 DAS.

Accession A_4 proved superior followed by A_1 for P, K and Ca contents at all the growth stages (Fig. 2). All the accessions were not significantly differ from each other for phosphorus content at 120 DAS (Fig. 2). A_4 and A_1 had the maximum content of protein (25.06 and 25.03%, respectively) and A_5 and A_2 (24.70 and 24.80%, respectively), followed by A_3 (24.86%) had less protein content, which had almost equal value (Fig. 2). On the other hand, A_4 surpassed the others for carbohydrate content and had the maximum value (53.16%), whereas, A_5 reported the minimum (50.62%). As far as tyrosinase activity is concerned, accession A_4 produced the maximum activity for tyrosinase and A_5 gave the minimum value (Fig. 2).

DISCUSSION

The present study indicates that differences among hyacinth bean accessions in these agro-botanical attributes exist. Accession A_4 proved superior over all other accessions and significant

differences ($p \leq 0.05$) for most attributes were observed. Variation in biomass production among hyacinth bean accessions due to accessions phenology, environment and season has been reported in various accessions (Holland and Mullen, 1995; Bernas, 1996; Murphy and Colucci, 1999; Pengelly and Maass, 2001; Ewansiha *et al*., 2007). According to agro-climatic conditions of India, intraspecific variations have been found on various physiological and morphological traits in other medicinal plants (Kulkarni *et al*., 1984; Singh *et al*., 1992). Furthermore, it has been reported that varietal differences among the accessions are greater than differences between related species or genera (Millikan, 1961). Significant differences in pod number, 100-seed weight and seed-yield among accessions was probably due to differences in their genetic makeup and the environmental conditions under which the hyacinth bean plants were grown. The higher values for nitrate reductase and carbonic anhydrase activities, net photosynthetic rate, stomatal conductance, nodule-nitrogen content together with appropriate amounts of nitrogen, phosphorus, potassium and calcium were also responsible for better hyacinth bean growth, number and dry weight of nodules. Hence, higher values of fresh and dry weights, number of pods and 100-seed weight of all accessions were recorded. Seed-yield is a cumulative performance of pod number, seed number per pod and 100-seed weight, respectively. Enhancement in yield attributes would ultimately culminate into seed-yield production of each accession. Similar studies regarding accession variation for different attributes in other plants were reported by Virk *et al.* (1989), Singh *et al.* (1992), Mishra *et al.* (2001), Choudhary and Gupta (2002), Khan *et al*. (2003), Naeem *et al.* (2006), Idrees *et al*. (2007) and Morris (2008).

In this study, the number and dry weight of nodules increased up to 90 DAS in all accessions (Table 1), however, the values decreased sharply thereafter. This is due to the fact that the initial competition for photosynthates was confined to roots, nodules and aerial vegetative organs. However, at 90 DAS, flowering and fruit setting provided strong sinks for the utilization of photosynthates. This created a shortage of photosynthates supply to the nodules leading to nodule degeneration as suggested by Samiullah and Khan (2003). The contents of chlorophyll and carotenoid were the optimum at 90 DAS in accession A_4. The chlorophyll content declined at later growth stages (Fig. 1). Reduction in total chlorophyll and carotenoid content might be due to the accelerated leaf-senescence as a result of ageing. Nitrate reductase and carbonic anhydrase were highest in accession A_4 at 60 DAS; however these activities decreased with increasing hyacinth bean age. Interestingly these activities were slower from the vegetative to flowering stage and more rapid from the flowering to fruiting stage in all accessions studied (Fig. 1).

As far as nitrogen-fixation in hyacinth bean accessions is concerned, accession A_4 generated the maximum number and weight of nodules, which requires phosphorus nutrition during the nodulation period. Actually, legumes require high amount of phosphorus for their growth, nodule formation and N_2-fixation. Leguminous crops have a high phosphorus utilization rate because of their greater requirement during nodulation (Carling *et al*., 1978) and N_2-fixation (Israel, 1987; Sonoboir and Sarawgi, 2000). The observed enhancement in number and dry weight of nodules due to phosphorus availability in the soil could be due to an additional fixation of molecular nitrogen leading to an improvement in nitrogen metabolism for hyacinth bean. The present study showed that nodule-nitrogen and leghaemoglobin contents declined with the age of the crop. Bacteria in the nodules depend upon the plants for their energy source. Therefore, prior to flowering, nodules can compete as a carbohydrate sink. However, once the plant enters the reproductive phase, seeds act as a stronger sink for carbohydrates than nodules, consequently the latter show a decreased dry nodule weight and lower nitrogen-fixing capacity.

Differences in mineral elements among other leguminous crops have been reported by Deka and Sarkar (1990), El Siddig *et al.* (2002) and Vadivel and Janardhanan (2002). Considering leaf-N, P, K and Ca contents at 90 and 120 DAS in hyacinth bean accessions, a decrease in the concentration of all these nutrients was noted, with the increase in the age of the hyacinth bean (Fig. 2). Such a decrease

in N, P, K and Ca contents of leaf may be due to a continuous utilization of these nutrients by the developing pods (sink) and their translocation from vegetative parts (source). Hyacinth bean seeds have higher values for carbohydrates when compared to groundnut (26.1%) and soybean (20.9%) (Narsinga Rao *et al*., 1989). This study shows that hyacinth bean has healthy mineral nutrients, seed-protein and carbohydrates for use as a high value grain, nutrient and green manure crop.

CONCLUSION

Hyacinth beans contain numerous and important therapeutic compounds for potential use in modern as well as traditional systems of medicine. This study proves that hyacinth bean seed is an important source of proteins, carbohydrates and minerals such as phosphorus, potassium and calcium. On the basis of present study conducted under the agro-climatic conditions of Aligarh, Western Uttar Pradesh, it is to be concluded that hyacinth bean (accession A_4) grew well in calcium deficient soil and can be cultivated in our country. Whereas, accession A_5 showed poorest performance under such conditions. Hyacinth bean has the potential to provide a quality and nutritious vegetable to the people of India.

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REFERENCES

Alhasan, S.A., O. Aranha and F.H. Sarkar, 2001. Genistein elicits pleiotropic molecular effects on head and neck cancer cells. Clin. Cancer Res., 7: 4174-4181.

Beckstrom-Sternberg, S.M. and J.A. Duke, 1994. The phytochemical database. Data Version July 1994. http://ars-genome.cornell.edu/cgi-bin/WebAce/webace?db=phytochemdb.

Bernas, P., 1996. Dry matter production and chemical composition of introduced forages at two moist savanna sites in Ghana. Trop. Grassland, 30: 418-421.

Carling, D.E., W.G. Riehle, M.F. Brown and D.R. Johnson, 1978. Effects of a vasicular-arbuscular mycorrhizal fungus on nitrate reductase and nitrogenase activities in nodulating and non-nodulating soybeans. Phytopathology, 68: 1590-1596.

Chopra, R.N., S.L. Nayar and I.C. Chopra, 1986. Glossary of Indian Medicinal Plants (Including the Supplement). 3rd Edn., Council of Scientific and Industrial Research, New Delhi.

Choudhary, S. and K. Gupta, 2002. Influence of seasons on biomass and alkaloid productivity in *Catharathus roseus*. J. Med. Arom. Plant Sci., 24: 664-668.

Cosetang, M.Y. and C.Y. Lee, 1987. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. J. Food Sci., 52: 985-989.

Deka, R.K. and C.R. Sarkar, 1990. Nutrient composition and antinutrional factors of *Dolichos lablab* seeds. J. Food Chem., 38: 239-246.

Dwivedi, R.S. and N.S. Randhawa, 1974. Evaluation of a rapid test for the hidden hunger of zinc in plants. Plant Soil., 40: 445-451.

El Siddig, O.O.A., A.H. El Tinay, A.W.H, Abd Alla and A.E.O. Elkhalifa, 2002. Proximate composition, minerals, tannins, *in vitro* protein digestibility and effect of cooking on protein fractions of hyacinth bean (*Dolichos lablab*). J. Food Sci. Technol., 39: 111-115.

Ewansiha, S.U., U.F. Chiezey, S.A. Tarawali and E.N.O. Iwuafor, 2007. Potential of *Lablab purpureus* accessions for crop-livestock production in the West African savanna. J. Agric. Sci., 145: 229-238.

Fiske, C.H. and Y. Subba Row, 1925. The colorimetric determination of phosphorus. J. Biol. Chem., 66: 375-400.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedure for Agricultural Research. 2nd Edn., John Wiley and Sons, New York, ISBN: 0-471-87931-2.

Hoffman, R., 1995. Potent inhibition of breast cancer cell lines by the isoflavonoid Kievitone: Comparison with genistein. Biochem. Biophy. Res. Commun., 211: 600-606.

Holland, J.F. and C.L. Mullen, 1995. *Lablab purpureus* (L.) Sweet (lablab) cv. Koala. Aust. J. Exp. Agric., 35: 559-559.

Idrees, M., M.M.A. Khan and M. Naeem, 2007. The superiority of rosea cultivar over alba of periwinkle (*Catharanthus roseus* L.) proved more potent source of alkaloids and other attributes. Turk. J. Biol. (In Press).

Israel, D.W., 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. Plant Physiol., 84: 835-840.

Jaworski, E.G., 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophy. Res. Commun., 43: 1247-1279.

Karachi, M., 1998. Growth and nutritive value of *Lablab purpureus* accessions in semi-arid Kenya. Trop. Grassland, 31: 214-218.

Khan, M.M.A., J. Puthukudi, F. Mohammad, M.H. Siddiqui and M. Naeem, 2003. Screening tomato varieties for phytonutrients productivity and yield performance. Muarik Bull., 6: 59-64.

Kirtikar, K.R. and B.D. Basu, 1995. Indian Medicinal Plants. Vol. I. 3rd Edn., Sri Satguru Publications, New Delhi.

Kobayashi, T., T. Nakata and T. Kuzumaki, 2002. Effect of flavonoids on cell cycle progression in prostate cancer cells. Cancer Lett., 176: 17-23.

Kulkarni, R.N., B.P. Dimri and K. Rajgopal, 1984. Variability for quantitative characters in periwinkle. Ind. Drug, 22: 61-64.

Lichtenthaler, H.K. and C. Buschmann, 2001. Extraction of photosynthetic tissues: Chlorophylls and carotenoids. Curr. Protocol. Food Anal. Chem., 1: F4.2.1-F4.2.6.

Lindner, R.C., 1944. Rapid analytical methods for some of the more common inorganic constituents of the plant tissues. Plant Physiol., 19: 76-89.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Maass, B.L., 2006. Changes in seed morphology, dormancy and germination from wild to cultivated hyacinth bean germplasm (*Lablab purpureus*: Papilionoideae). Genetic Res. Crop Evolu., 53: 1127-1135.

Matheis, G., 1987. Polyphenol oxidase and enzymatic browning of potatoes (*Solanum tuberosum*) I. Properties of potato polyphenol oxidase. Chem Mikrobiol. Technol. Lebensm, 11: 5-12.

Millikan, C.R., 1961. Plant varieties and species in relation to the occurrence of deficiencies and excess of certain nutrient elements. J. Aust. Inst. Agric. Sci., 26: 220-223.

Mishra, P., G.C. Uniyal, S. Sharma and S. Kumar, 2001. Pattern of diversity for morphological and alkaloid yield related trades among the periwinkle *Catharathus roseus* accessions collected from in and around Indian Subcontinent. Genetic Resour. Crop Evolu., 48: 273-286.

Morris, J.B., 1996. Special legumes may be an unopened medicinal chest. Agric. Res. November, pp: 13-15.

Morris, J.B., 1997. Special-purpose legume genetic resources conserved for agricultural, industrial and pharmaceutical use. Econ. Bot., 51: 251-263.

Morris, J.B., 1999. Legume Genetic Resources with Novel Value Added Industrial and Pharmaceutical Use. In: Perspectives on New Crops and New Uses, Janick, J. (Ed.). ASHS Press, Alexandria, VA., pp: 196-200.

Morris, J.B., 2003. Bio-functional legumes with nutraceutical, pharmaceutical and industrial uses. Econ. Bot., 57: 254-261.

Morris, J.B., 2008. *Macrotyloma axillare* and *M. uniflorum*: Descriptor analysis, anthocyanin indexes and potential uses. Genetic Resour. Crop Evolu., 55: 5-8.

Murphy, A.M. and P.E. Colucci, 1999. A tropical forage solution to poor quality diets: A review of *Lablab purpureus*. Livestock Res. Rural Dev. 11 (2).

Naeem, M., F. Mohammad, M.M.A. Khan, M.H. Siddiqui and I. Ahmad, 2006. A pot test evaluation of the growth and yield of seven carrot cultivars. Test Agrochem. Cultiv., 27: 11-12.

Narsinga Rao, B.S., Y.G. Deosthle and K.C. Pant, 1989. Nutritive Value of Indian Foods. 1st Edn., India Council of Medical Research, National Institute of Nutrition, Hyderabad.

Paul, B. and L.R. Gowda, 2000. Purification and characterization of a polyphenol oxidase from the seeds of field Bean (*Dolichos lablab*). J. Agric. Food Chem., 48: 3839-3846.

Pengelly, B.C. and B.L. Maass, 2001. *Lablab purpureus* (L.) sweet-diversity, potential use and determination of a core collection of this multi-purpose tropical legume. Genetic Res. Crop Evolu., 48: 261-272.

Sadasivam, S. and A. Manickam, 2008. Biochemical Methods. 3rd Edn., New Age International Publishers, New Delhi, India, ISBN-13: 9788122421408.

Samiullah and N.A. Khan, 2003. Physiological investigations on interactive effect of P and K on growth and yield of chickpea. Ind. J. Plant Physiol., 8: 165-170.

Shivashankar, G. and R.S. Kulkarni, 1989. Field bean (*Dolichos lablab* Linn. var. Lignosus Prain). Indian Hortic., 34: 24-27.

Singh, B.M., S.K. Pareek, S. Mandal, M.L. Maheshwari and R. Gupta, 1992. Variability in periwinkle (*Catharanthus roseus*). Ind. J. Agric. Sci., 62: 47-50.

Sonoboir, H.L. and S.K. Sarawgi, 2000. Nutrient uptake, growth and yield of chickpea as influenced by phosphorus, rhizobium and phosphate solubilizing bacteria. Madras Agric. J., 87: 149-151.

Subba Rao, N.S., 1972. Bacterial culture boosts pulse yields. Intensive Agric., 10: 2-3.

Sánchez-Ferrer, A., J.N. Rodríguez-López, F. García-Cánovas and F. García-Carmona, 1995. Tyrosinase: A comprehensive review of its mechanism. Biochim. Biophys. Acta., 1247: 1-11.

Vadivel, V. and K. Janardhanan, 2002. Agrobotanical traits and chemical composition of *Cassia obtusifolia* L.: A lesser-known legume of the Western Ghats of South India. Plant Foods Hum. Nutr., 57: 151-164.

Virk, S.S., O.S. Singh and B.S. Bhullar, 1989. Studies on diversity in periwinkle. Crop Improv., 16: 49-52.

Watson, D.J., 1958. The dependence of net assimilation rate on leaf-area index. Ann. Bot., 22: 37-54.