Variation in nutrient acquisition and utilization efficiency of grasspea genotypes

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Abstract

Grasspea (*Lathyrus sativus* L) is grown as pulse and fodder crop in many parts of the developing world and it is well adapted to extreme drought, water-logging and nutrient-stress conditions. Little is known about the genetic diversity in root traits and nutrient uptake of grasspea. Root traits (root length, root hairs, root-induced acidification and phosphatase enzymes) of five grasspea genotypes (Khesari-1, Khesari-2, Jamalpur Local, Cgi-8931144 and Cgi-8431418) were investigated in relationship to the efficiency of nutrient acquisition (NAE, nutrient acquired per unit root length) and nutrient utilization (NUE, amount of shoot biomass produced per unit nutrient acquired) of Phosphorus (P) Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S), Iron (Fe), Manganese (Mn), Zink (Zn), Copper (Cu), Boron (B), Molybdeum (Mo) and Cobalt (Co) in laboratory and pot experiments. The genotypes differed significantly ($p<0.05$) in root length (RL), root-shoot ratio (R: S) and in NAE and NUE. The improved varieties, Khesari-1 and Khesari-2, produced larger RL and had higher R: S, compared to the other genotypes. The genotypes did not differ in root hair formation and they also did not differ to induce rhizosphere acidification and acid phosphatase activity (Aptase). Khesari-1 and Khesari-2 were in general superior (high NAE) to mobilize and acquire K, Mg, S, Fe and Mn, which was related to their higher R: S. Local and Cgi-8431418 were in general superior to utilize most of the nutrients more effectively to produce shoot biomass, as indicated by the high values for NUE. It is suggested that for searching nutrient-efficient grasspea germplasm, both NAE and NUE should be considered equally important.

Introduction

Grasspea is widely cultivated in the tropical as well as in the temperate countries, Mediterranean Basin, Ethiopia, India, Bangladesh and Central Asia (Kislev, 1989) for grain, fodder, hay and green manuring. It has a prostrate growth habit, with thin grass-like foliage and red-brown, blue or white coloured flowers. It is an annual legume, well adapted to low rainfall, but it is also fairly tolerant to water-logging (Rahman et al., 1995) and it is often the last crop to stand in case of extreme conditions. Though grasspea seeds contain a neurotoxic compound ODAP (b-N-Oxalyl-diaminopropionic acid) potentially causing lathyrism (irreversible paralysis of lower limbs) in human beings, it is widely cultivated because of its wide adaptability and little requirement for inputs.

L. sativus (grasspea) is an important pulse crop in India, Bangladesh, China, Pakistan and Nepal (Campbell et al., 1994; Yadav and Mehta, 1995). Research has suggested the possibilities to breed low toxin varieties, relatively safe for human consumption. In the past little effort has been expended towards selection and breeding for improvement of the grasspea as a food crop, due to its utilisation as forage crop (Smartt, 1984). However, the grasspea has potential as an alternative pulse in many cropping systems around the world including Australia (Siddique et al., 1996). To promote research addressing the aspects of improvement of the grasspea, including low concentrations of ODAP in the seed and higher yield (Campbell et al., 1994), it is important to assess the intra-specific variation for exploring the potential for genetic improvement.

Despite the good knowledge of the fact that grasspea adapts well both to drought and water-logging conditions, the knowledge on genetic diversity in root system of grasspea is rudimentary. Even less is known about whether genetic diversity in grasspea root traits is related to better capture of soil nutrients.

For higher plants the essentiality of 15 mineral elements mainly absorbed from soil by roots is well documented (Marschner, 1995). Co is considered essential for nodule development and nitrogen fixation by legumes (Dilworth and Bisseling, 1984). Grasspea, a legume, may partial satisfy its need for nitrogen (N) when it is fixing atmospheric nitrogen with the help of root associated *Rhizobium.* Deficiency of any one of the other nutrient elements may limit its growth and economic output (Marschner 1995). Therefore, an integrated approach to explore genetic diversity in as many root traits and to assess their role in uptake of various nutrient elements is helpful to understand the reasons for its wide adaptability. From mineral nutrition point of view, a genotype may be more nutrient-efficient than others if it mobilizes and absorbs more nutrient from soils (Nutrient Acquisition Efficiency, NAE, expressed as amount of nutrient acquired per unit root length) and/or makes better utilization of

the absorbed nutrient to produce biomass (Nutrient Utilization Efficiency, NUE, expressed as amount of biomass produced per unit of nutrient accumulated in the biomass).

To understand the role of grasspea root traits in uptake of the various nutrient elements and to explore the genetic diversity, root morphological (root length, root hairs) and physiological (root exudation of protons and phosphatase enzymes) traits were investigated in relation to the NAE and NUE of five selected varieties/breeding lines of grasspea for twelve minerals elements (except N, Cl and Na).

Materials and Methods

Genotypes

Five varieties/breeding lines (genotypes) of grasspea *(Lathyrus sativus L.)* were selected for investigation; based on the popularity among Bangladeshi farmers and the anticipated importance for breeding new improved varieties. Khesari-1 and Khesari-2 are the improved varieties, `Jamalpur` (Local) is a landrace and Cgi-8931144 and Cgi-8431418 are breeding lines of potential importance.

Soil properties

Some properties of soil used in the pot experiment are the following,

Soil pH 7.7 (0.01 *M* $CaCl_2$); organic matter 0.55 %; total N 0.029 %; major cations extracted with ammonium acetate and measured with flame photometer (Doll and Lucas, 1973) (meq/100ml), Ca = 12.0; Mg = 2.5; K = 0.25 and other nutrients (µg/g) P = 10.3 (Olsen et al., 1954) ; S = 20 (Tabatabai, 1982); B = 0.59 (hot water extract; Bingham, 1982); Cu = 6.3; Fe = 11; Mn = 6; Zn = 1.7 (DTPA extracted and measured with atomic absorption spectroscopy (Lindsay and Norvell, 1978).

Determination of root growth and length

The shoot growth and root length (RL) of the genotypes were studied in a pot experiment at Pulses Research Center, Ishurdi, Bangladesh. Pots were made by cutting two-litre transparent plastic bottles (Figure 1A). They were filled with 2.2 kg of soil by shaking to achieve soil bulk density of 1.4 g cm^{-3}. The soil columns of all the pots were 25 cm high. The pots were placed in the open, sides wrapped in black polythene to prevent exposure of roots to light and maintained at 20 % soil moisture by weighing and adding water. Six seeds were sown at 1-cm soil depth. At germination (3-4 days after sowing), three seedlings were left in each pot by removing the rest of the seedlings along with the roots. There were four replicates. At 20 and 60 days after sowing (20 DAS and 60 DAS respectively), the shoots were cut and stored in paper bags for drying and determination of relative growth rate (RGR) and nutrient analyses. The plastic pots were cut opened; the roots were washed out of soil and cleaned off debris. Pots were not inoculated, but minor nodulation was observed in all pots at 60 DAS.

Visual assessment of the washed out roots gave an idea about that no differences in nodulation existed among the genotypes. One g of fresh root sample was spread between polythene transparencies and scanned using ScanJet IIcx. The total length of the root system was measured using *Dt-Scan software* (Delta-T Devices, Cambridge, England) as described in Gahoonia et al., (1999).

Plant analyses

Shoots at flowering stage (60 DAS) were dried at 60°C until constant weight was recorded. The whole plant material of each pot was ground using an Ultra Centrifugal Mill (Retsch ZM 100). Plant material (0.25 g) was digested in an open vessel system using 70 ml HD polyethylene vials (Capitol Vial Corp, Fulton Ville, NY, USA) using a graphite-heating block (Mod Block, CPI International, Amsterdam, Holland). The plant material was digested at 95°C using a slight modification of the EPA (Environmental Protection Agency, USA) Method 3050B, as described below. Five ml of 35% HNO_3 (Instra analysed, Baker, Deventer, Holland) was added to the samples and the samples were boiled for approximately 15 minutes. After cooling 2.5 ml 70% HNO_3 was added and the samples were reheated. Twenty five minutes later samples was cooled and 1.5 ml H_2O_2 (Extra pure, Riedel-de Haën, Seelze, Germany) was applied. When the peroxide reaction ceased, 1 ml of H_2O_2 was added and samples were reheated for approximately 40 minutes. During the digestion, vials were covered by watch glasses. Samples were cooled overnight and diluted to 50 ml with ultra pure water. For each digestion five blank samples were included. Furthermore samples of a certified reference material-CRM (Apple leaf, standard reference material 1515; National Institute of Standards and Technology, Gaithersburg, MD, USA) were digested to estimate the accuracy and precision of the analysis. Finally, an in house barley reference material was included in order to keep a check of element concentrations in each individual run on the ICP-MS. Samples were diluted to the same acid concentration (1.75% HNO_3) as standards and quantification was done by external calibration (P/N 4400 ICP-MS, Multi-elemental calibration standard, CPI-International, Amsterdam, Holland). Dilutions were performed in a class 100 laminar flow bench (KR-170s Biowizard, Kojair Tech Oy, Vilppula, Finland).

Twelve elements (K, P, Ca, Mg, S, Fe, Zn, Mn, Cu, B, Mo, Co) were analysed by ICP-MS (Agilent 7500c, Agilent Technologies; Manchester, England). Nitrogen was not analysed, because grasspea, a legume, can fix and make use of atmospheric N_2 and N uptake is less dependent on size of the root system (Atkinson, 1992).

Determination of root hairs

The soil was filled in 10-cm long test tubes of diameter 3 cm (soil bulk density 1.4 g cm^{-3}, soil moisture 20 %, four replicates). One pre-germinated seed was planted in each tube. After 20 days, the

tubes, after cutting the shoot, were immersed in water overnight in a dark room to prevent mucilage formation. All roots were removed carefully using a kitchen sieve and transferred into an Ultrasound water bath (Branson 5200, 120W, 47k Hz). The ultrasound treatment for about 5-10 minutes removed remaining soil particles without damaging the root hairs. The root hairs were quantified using Quantimet 500+ Image Processing and Analysis System (Leica) at 10x magnification (Gahoonia and Nielsen, 1997).

Determination of rhizosphere pH

The roots of 10 days old seedlings were embedded in agar containing pH indicator dye *Bromocresol purple* and adjusted to pH 6 (Marschner and Römheld, 1983). The root-induced pH change, revealed by colour change, was recorded after one hour.

Rhizosphere phosphatase activity

The ability of the genotypes to release acid phosphatase (Aptase) in the rhizosphere was determined by the method of Dinkelaker and Marschner (1992), which is based on enzymatic hydrolysis of 1-naphtylphosphate (substrate) by root-released Aptase, yielding 1-naphtol, which produces a red complex with Fast Red TR (dye). The intact roots of 10 days old seedlings were sand-wiched between two ashless filter papers, soaked in a mixture of the dye and the substrate. If roots release variable amounts of phosphatase enzymes, their activity is visible as reddish brown colour of variable intensity near the roots, because root-released phosphatase produces reddish brown complex with the dye Fast Red TR.

Statistical analyses were performed with Statistical Analysis System (SAS) Institute (1989) and Microsoft Excel software as found appropriate. Statistical significant ($p<0.05$) differences between the genotypes was analysed by analysis of variance (ANOVA).

Results

The growth patterns of the selected genotypes did not differed, except that shoot dry matter (DM) of Khesari-1 was significantly ($p<0.05$) lower that other genotypes (Figure 2).

Root traits

There were significant ($p<0.05$) differences in root length (RL) of the genotypes after 20 DAS (Figure 3). The improved varieties Khesari-1 and Khesari-2 produced larger RL at flowering (60 DAE) and other genotypes did not differ in their RL (Figure 1 B; Figure 3). The root to shoot ratio (R: S) was highest in case of Khesari-1 (3.10 cm root mg^{-1} DM), followed by Khesari-2 (2.70 cm root mg^{-1} DM). The landrace Local (1.90 cm root mg^{-1} DM) had lowest R: S value (Table 3).

The roots of Khesari-2 were covered with longest root hairs (0.50 ± 0.11 mm, Figure 4), followed by Kheasri-1 (0.38± 0.12 mm) and Local (0.31± 0.10 mm), but genotypic differences in root hair length (RHL, Figure 4) and root hair density (RHD, Figure 1C and 1D) were not significant ($p<0.05$).
The colour indicator dye methods did not reveal/detect differences in root-induced rhizosphere pH and acid phosphatase activity (Aptase) in the rhizosphere of the selected genotypes (Data not shown).

Macronutrients

Potassium (K)

The concentration of K (Table 1) was highest in the shoot biomass of Khesari-2 (25.7 g kg^{-1} DM), followed by Khesari-1 (24.6 g kg^{-1} DM). The variation in K uptake of the genotypes was significant ($p< 0.05$). The K acquisition efficiency (NAE = mg K m^{-1} root length, RL) of Khesari-1 and Cgi-8931144 and Local were superior (0.71) compared to Khesari-2 (0.64) and Cgi-8431418 (0.52) (Table 3) and the genotypic differences were significant ($p<0.05$). The K utilization efficiency (NUE = g DM produced mg^{-1} nutrient in DM) of K was highest for Cgi-8431418 (138), followed by Cgi-8931144 (121). Other genotypes produced significantly ($p<0.05$) less DM per mg^{-1} of K (Table 4).

Phosphorus (P)

Local landrace accumulated highest amount (3.4 g kg^{-1}) of P in its DM, followed by Cgi-8931144 (2.8 g kg^{-1}) (Table 1) and they were able to acquire more P per unit RL (NAE = 0.113 mg m^{-1}), followed by Cgi-8931144 (NAE = 0.095 mg m^{-1} RL) as compared to other genotypes (Table 3). The NUE of genotype Cgi-8431418 (1081 g DM g^{-1} P) was considerably higher than Khesari-1 (486 g DM g^{-1} P) and the genotypic differences were significant ($p<0.05$) (Table 4).

Calcium (Ca)

The highest amount of Ca was in the DM of Local (11.1 g kg^{-1}), fallowed by Cgi-8931144 (8.3 g kg^{-1}). Other genotypes did not differ in the amount of Ca in their DM (Table 1). The NAE of Khesari-1 was highest (32 mg Ca m^{-1} RL) and that of Khesari-2 the lowest (19 mg Ca m^{-1} RL), other genotypes taking the intermediate ranking (Table 3). Utilization efficiency (NUE) of Ca differed significantly ($p<0.05$) between the genotypes; Local produced 348 g DM g^{-1} Ca and Khesari-1 produced 114 g DM g^{-1} of Ca accumulated (Table 4).

Magnesium (Mg)

Khesari-2 absorbed highest amount of Mg (2.6 g kg^{-1}), followed by Khesari-1 (2.5 g kg^{-1}). Other genotypes did not differ to absorb and accumulate Mg in their DM (Table 1). Mg acquisition efficiency was the highest (0.072 mg Mg m^{-1} RL) with Khesari-1 and the lowest (0.053 mg Mg m^{-1} RL) with Cgi-8431418 (Table 3). When the ability of the genotypes to produce biomass per unit of

Mg accumulated (NUE) is compared, Cgi-8431418 produced 1369 g DM g^{-1} Mg and Khesari-1 produced 504 g DM g^{-1} Mg accumulated (Table 4). The genotypic differences in NUE of Mg were significant ($p<0.05$).

Sulphur (S)

The improved varieties Khesari-1 (3.9 g S kg^{-1}) and Khesari-2 (3.6 g S kg^{-1}) accumulated significantly ($p<0.05$) higher amount of S in their DM as compared to other genotypes (Table 1). Khesari-1 together with Local acquired higher amount of S (11 mg m^{-1} RL) as compared to other genotypes (Table 3). The ability of the genotypes to produced biomass per unit of S accumulated (NUE = g DM g^{-1} S) ranged between 1004 (Cgi-8431418) and 321 (Khesari-1) (Table 4).

Micronutrients

Iron (Fe)

There was a significant ($p<0.05$) variation among the genotypes in Fe uptake. Improved varieties Khesari-2 (328 mg Kg^{-1} DM) and Khesari-1 (304 mg kg^{-1} DM) absorbed significantly higher amount Fe in their DM compared to other genotypes (Table 2). The Fe acquisition efficiency (NAE = µg Fe m^{-1} RL) of Khesari-1 (8.73) and Khesari-2 (8.12) was higher (Table 3) than other genotypes e.g. Cgi-8431418 (6.73). The Local showed highest (12 g DM mg^{-1} Fe in DM) Fe utilization efficiency (NUE), followed by Cgi-8431418 (11 g DM mg^{-1} Fe in DM). The NUE of Fe in case of the improved varieties Khesari-1 (4 g DM mg^{-1} Fe in DM) and Khesari-2 (7 g DM mg^{-1} Fe in DM) was low (Table 4).

Manganese (Mn)

Kheasri-1 and Khesari-2 absorbed 65.8 mg Mn kg^{-1} DM and 56.4 mg Mn kg^{-1} DM respectively. These values were significantly higher than those of other genotypes (Table 2). The values of NAE was highest with Khesari-1 (1.89 µg Mn m^{-1} RL) and lowest with Cgi-8431418 (1.06 µg Mn m^{-1} RL); other genotypes having intermediate values (Table 3). The Mn utilization efficiency of Cgi-8431418 was highest (68 g DM mg^{-1} Mn) and that of Khesari-1 was lowest (19 g DM mg^{-1} Mn) (Table 4).

Zinc (Zn)

The Zn uptake was highest with Local (14.4 mg kg^{-1} DM), followed by Cgi-8931144 (12.4 mg kg^{-1} DM). The Zn uptake of the improved varieties, Khesari-1 and Khesari-2 was lower than other genotypes (Table 2). The genotypic differences in Zn uptake were significant ($p<0.05$). As shown in Table 3, the NAE for Zn was highest with Local (0.47µg Zn m^{-1} RL) and lowest with Khesari-2 (0.22µg Zn m^{-1} RL). The genotype Cgi-8431418 used one mg of Zn to produce 193 g DM, whereas Khesari-2 produced only 126 g DM with the same amount of Zn accumulated (Table 4).

Copper (Cu)

The Cu uptake of the investigated genotypes varied significantly ($p<0.05$), with Local absorbing highest (9.3 mg kg^{-1} DM) and Cgi-8431418 absorbing the lowest (7.5 mg kg^{-1} DM) amount of Cu (Table 2). The Cu acquisition efficiency of Local was 0.31µg m^{-1} RL and that of Cgi-8431418 was 0.24µg m^{-1} RL (Table 3). These two genotypes, Local (300 g DM mg^{-1}) and Cgi-8431418 (272 g DM mg^{-1}) were also able to utilise the accumulated Cu more efficiently to produce DM compared to other genotypes (Table 4).

Boron (B)

There were significant ($p<0.05$) differences in B uptake of the investigated genotypes. Cgi-8431418 absorbed highest (13.4 mg kg^{-1} DM) and Khesari-1 the lowest (10.1 mg kg^{-1} DM) amount of B (Table 2), which corresponded to their highest NAE (0.43 µg m^{-1} RL with Cgi-8431418) and lowest NAE (0.29 µg m^{-1} RL with Khesari-1) (Table 3). The B utilization efficiency was the highest with Local and Cgi-8931144 (203 and 202 g DM mg^{-1} B respectively) and the lowest (126 g DM mg^{-1} B) with Khesari-1 (Table 4).

Molybdenum (Mo)

The uptake of Mo was the highest (0.85 mg kg^{-1} DM) with Local and the lowest (0.71 mg kg^{-1} DM) with Cgi-8431418 (Table 2) and there was significant ($p<0.05$) variation in Mo uptake of other genotypes. The higher Mo uptake by Local corresponds to its ability to acquire higher amount (0.028 mg Mo m^{-1} RL) of Mo per unit root (NAE) as compared to other genotypes (Table 3). Both Local and Cgi-8431418 also showed higher utilization efficiency of Mo and they were able to produce 3200 and 2997 g DM mg^{-1} Mo respectively (Table 4) in their biomass.

Cobalt (Co)

Most of the genotypes absorbed Co in the range of 0.20 mg kg^{-1} (Khesari-1) and 0.27 mg kg^{-1} (Cgi-8431418) (Table 2) but its acquisition efficiency did not differ ($p<0.05$) with genotype (Table 3).

Discussion

The relative growth rate (RGR) of the genotypes was 0.13±0.02, indicating a moderate nutrient stress conditions (Rakhmankulova et al., 2001) in the pot experiment. There were non-significant differences in shoot dry matter (DM) and RGR among the genotypes, except in case of Khesari-1 (Figure 1). The investigated grasspea genotypes differed significantly ($p<0.05$) in root length (RL) (Figure 2), root-shoot ratio (R: S) and in uptake of plant nutrients (Table 1). Although no information specific to the nutrient uptake of grasspea could be found in the existing literature, the concentration range of the

most elements (except Fe and Mn) lies close to lower limit of the critical deficiency levels (Bergmann, 1992; Marschner, 1995) in DM generally reported for legumes. The results showed significant genotypic variation in the efficiency to acquire (NAE) as well as to utilize (NUE) nutrients by the grasspea genotypes. Gourley et al. (1994) proposed that germplasm may be described to differ in nutrient efficiency only when similar yields are obtained. In our experiments the most genotypes did not differ to produce DM (Figure 1) and their RGRs were almost identical.

The improved varieties Khesari-1 and Khesari-2 were in general superior to mobilize and acquire K, Mg, S, Fe and Mn (Table 1), which seemed linked to their higher root to shoot ratio (Table 3). Local, a landrace, was in general able to utilize most of the nutrients more effectively to produce shoot biomass, as indicated by high values for Nutrient Utilization Efficiency (Table 4). The abilities of the breeding lines to acquire nutrients (NAE) were not outstanding, though Cgi-8431418 was able to utilize most of the nutrients more effectively than other genotypes as indicated by relatively high values for its NUE (Table 4). In case of many nutrients, the genotypes which showed higher NAE (amount of nutrient m^{-1} RL) had lower NUE (g DM mg^{-1} nutrient absorbed (compare Table 3 and Table 4). Hence the some genotypes appeared more efficient to utilization nutrients (more biomass with less nutrient content) merely because they were inefficient to obtain nutrients from soils (Caradus, 1991). If we concentrate on nutrient utilization efficiency (amount of dry matter produced per unit of nutrient absorbed) without giving due attention to the nutrient acquisition efficiency, the selection and breeding approaches may result in production of lush feed/grain that is too low in macro- and micro-nutrients for animal/human health. Due to lower concentration of nutrients in their tissues and grains, their nutritive value may be lower than conventional cultivars.

A number of root traits may influence nutrients from soil by the genotypes (Gahoonia and Nielsen, 2004). Root architecture and placement of roots in the soil profiles play an important role in nutrient capture and plant productivity (Lynch, 1995). The size and architecture of root system may depend on the formation of lateral roots (Mia et al., 1996). In the pot experiment, the genotypic variation in lateral spread of the roots in soil profiles could not be determined. The enlargement of effective root lengths of the genotypes due to the differential presence of root hairs may also affect uptake of nutrients (Gahoonia and Nielsen, 2003). However, the root hair formation of the investigated grasspea genotypes did not differ significantly (Figures 1C and 3).

Plant species, especially nodule forming and nitrogen fixing legumes, possess the potential to induce rhizosphere acidification (Tang et al., 1997), but the differences in rhizosphere pH were not detected among the investigated grasspea genotypes. Root induced rhizosphere pH is known to influence

availability of soil inorganic phosphorus (Gahoonia and Nielsen, 1992) and micronutrients to plants (Marschner and Römheld, 1996). The investigated grasspea genotypes did not show differences in rhizosphere activity of phosphatase enzymes, suggesting that the observed variation in P uptake of the genotypes may not be due to the mobilisation of soil organic phosphorus (Asmar et. al., 1995; Li et al., 2004). A number of other factors like root-released organic acids (Ryan et al., 2001); change in rhizosphere redox potential for Fe and Mn acquisition, especially under water-logging conditions (Shiferaw et al., 1992); rhizosphere microorganisms (Zaidi et al., 2003) and mycorrhizae (Alloush et al., 2000; Weber et al., 1992) may play a role in acquisition of soil nutrients. In the present study the potential role of these factors was not investigated. Integration of these factors in future studies for identification of nutrient-efficient grasspea germplasm would be beneficial to understand its adaptability in wide range of environments. Although in the present study the genotypes did not appear to differ in nodulation, the studies with other legumes (Unkovich and Pate, 2000) indicate that such variation may exist. The exploration of genetic variation in the ability of nitrogen fixation among the grasspea genotypes/landraces through more detailed studies, therefore, deserves a special attention Further investigations will be needed to obtain more information on the role of these factors to understand the adaptation of grasspea to adverse conditions.

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Table 1 Concentration of macronutrients in shoot dry matter (DM) of five grasspea genotypes. (Mean ± standard error of means, n= 4).

Genotypes	macronutrients (g kg^{-1} DM)				
	K	P	Ca	Mg	S
Khesari-1	24.6±1.7	2.6±0.03	11.1±1.1	2.5±0.03	3.9±0.10
Khesari-2	25.7±1.6	2.6±0.10	7.6±0.2	2.6±0.10	3.6±0.20
Local	21.1±0.5	3.4±0.10	7.3±0.2	1.9±0.1	3.2±0.11
Cgi-8931144	20.8±1.3	2.8±0.10	8.3±0.1	1.8±0.2	2.9±0.15
Cgi-8431418	16.3±1.0	2.1±0.10	7.3±0.1	1.7±0.03	2.2±0.13

Table 2 Concentration of micronutrients in the shoot dry matter (DM) of five grasspea genotypes. (Mean ± standard error of means, n= 4).

Genotypes	micronutrients (mg kg^{-1} DM)						
	Fe	Mn	Zn	Cu	B	Mo	Co
Khesari-1	304 ± 4	65.8±4	10.0±0.6	8.3±0.1	10.1±0.4	0.78±0.01	0.20±0.01
Khesari-2	328±4	56.4±6	8.8±0.2	8.3±0.3	12.4±0.3	0.78±0.02	0.23±0.02
Local	221±5	45.4±2	14.4±2.2	9.3±0.5	12.5±0.4	0.85±0.04	0.20±0.01
Cgi-8931144	232±1	44.2±2	12.4±0.1	8.0±0.3	10.9±0.7	0.79±0.03	0.21±0.01
Cgi-8431418	210±5	33.1±2	11.9±1.3	7.5±0.2	13.4±1.3	0.71±0.01	0.27±0.03

Table 3 Root shoot ratio (R: S = cm of root per mg of shoot DM) and nutrient acquisition efficiency, expressed as amount of nutrient absorbed by unit length of root, of macro- and micro-nutrients in shoot dry mater (DM) of five grasspea genotypes. (Mean ± standard error of means, n= 4).

Nutrients	Genotypes				
	Khesari-1	Khesari-2	Local	Cgi-8931144	Cgi-8431418
R: S	3.10±0.18	2.70±0.23	1.90±0.03	2.10±0.13	2.10±0.16
Macronutrients					
(mg m^{-1} root)					
	Khesari-1	Khesari-2	Local	Cgi-8931144	Cgi-8431418
K	0.71±0.08	0.64±0.08	0.70±0.04	0.71±0.04	0.52±0.01
P	0.071±0.005	0.064±0.007	0.113±0.008	0.095±0.016	0.066±0.001
Ca	0.32±0.05	0.19±0.01	0.24±0.02	0.29±0.04	0.24±0.01
Mg	0.072±0.004	0.064±0.002	0.064 ±0.004	0.062±0.001	0.053±0.003
S	0.11±0.01	0.08± 0.02	0.11±0.01	0.10±0.01	0.07±0.01
Micronutrients					
(µg m^{-1} root)					
	Khesari-1	Khesari-2	Local	Cgi-8931144	Cgi-8431418
Fe	8.73±0.54	8.12±0.64	7.36±0.91	7.98±0.95	6.73±0.06
Mn	1.89±0.19	1.40±0.23	1.51±0.21	1.52±0.23	1.06±0.04
Zn	0.29±0.03	0.22±0.01	0.47±0.02	0.43±0.05	0.38±0.06
Cu	0.24±0.01	0.21±0.01	0.31±0.02	0.27±0.03	0.24±0.01
B	0.29±0.01	0.31±0.03	0.42±0.06	0.37±0.07	0.43±0.06
Mo	0.022±0.001	0.019 ±0.002	0.028±0.003	0.027±0.004	0.023±0.001
Co	0.005±0.001	0.006±0.001	0.006±0.002	0.007±0.001	0.007±0.001

Table 4 Nutrient utilization efficiency, expressed as biomass produced per unit of nutrient accumulated, of macronutrients and micronutrients accumulated in shoot dry mater (DM) of five grasspea genotypes. (Mean ± standard error of means, n= 4).

Nutrients	Genotypes				
	Khesari-1	Khesari-2	Local	Cgi-8931144	Cgi-8431418
Macronutrients (g DM g^{-1} nutrient)					
K	52±3	88±2	121±18	94±5	138±22
P	486±1	873±2	742±102	707±111	1081±197
Ca	114±10	297±18	348±50	234±27	307±73
Mg	504±2	874±70	1318±176	1076±2 5	1369±326
S	321±15	675±67	807±164	660±37	1004±165
Micronutrients					
(g DM mg^{-1} nutrient)					
	Khesari-1	Khesari-2	Local	Cgi-8931144	Cgi-8431418
Fe	4±0.2	7±0.2	12±2	8±1	11±2
Mn	19±1	40±3	56±12	44±6	68±10
Zn	126±9	255±15	176±3	157±18	193±63
Cu	152±1	270±2	272±33	243±25	300±74
B	126±6	182±3	203±29	202±31	171±53
Mo	1630±10	2909±33	2997±558	2473±353	3200±738
Co	6664±396	10085±1338	13093±2692	9365±1624	10450±1972

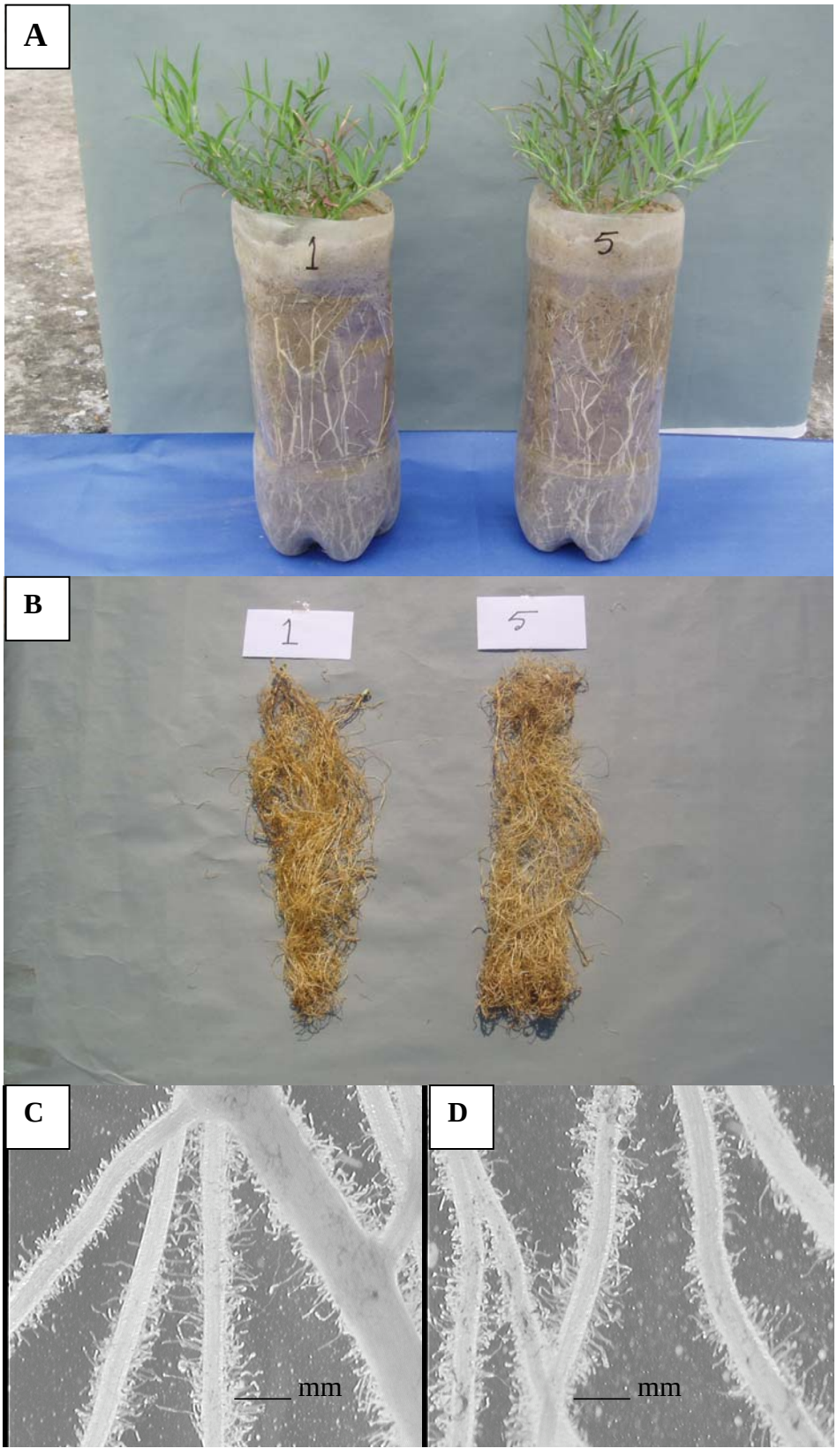
Capture for Figures

Figure 1 Shoot and root growth of two grasspea varieties, Khesari-1 and Khesari-2. Pots with visible roots of Khesari-1 (1) and Khesari-2 (5), 60 days after sowing (A). Root system of Khesari-1(1) and kheasri-2 (5), 60 days after sowing (B). Root hairs on the roots of Khesari-1(C) and Khesari-2 (D).

Figure 2 Shoot biomass of five grasspea genotypes 60 days after sowing in a pot experiment. Bars are standard error of means (n = 4).

Figure 3 Root lengths of five grasspea genotypes 20 days after sowing (20 DAS) and 60 days after sowing (60 DAS). Bars are standard error of means (n = 4).

Figure 4 Average root hair length of grasspea genotypes, Khesari-1, Khesari-2 and Local landrace. Bars are standard error of means (n = 4).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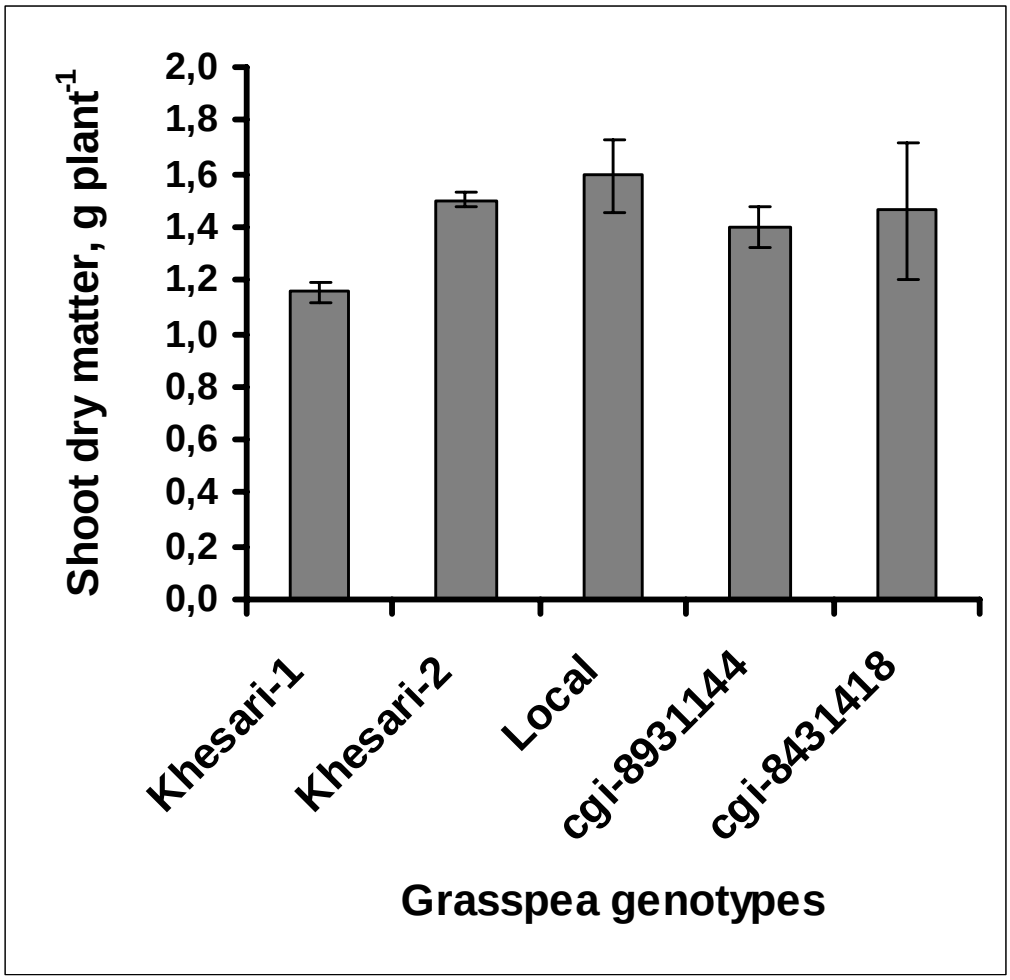

Figure 2

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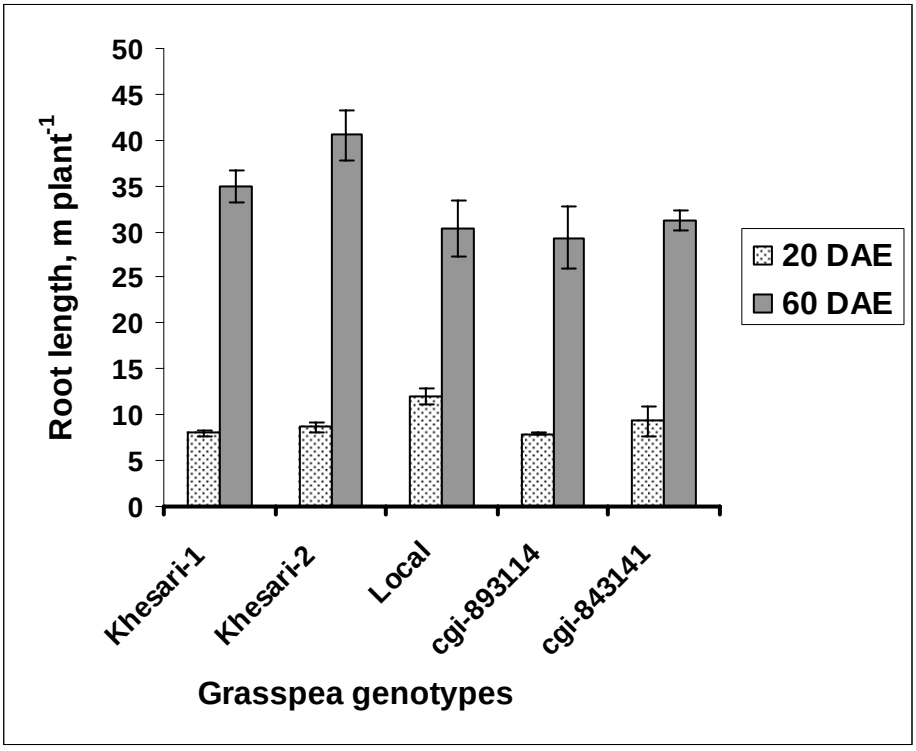

Figure 3

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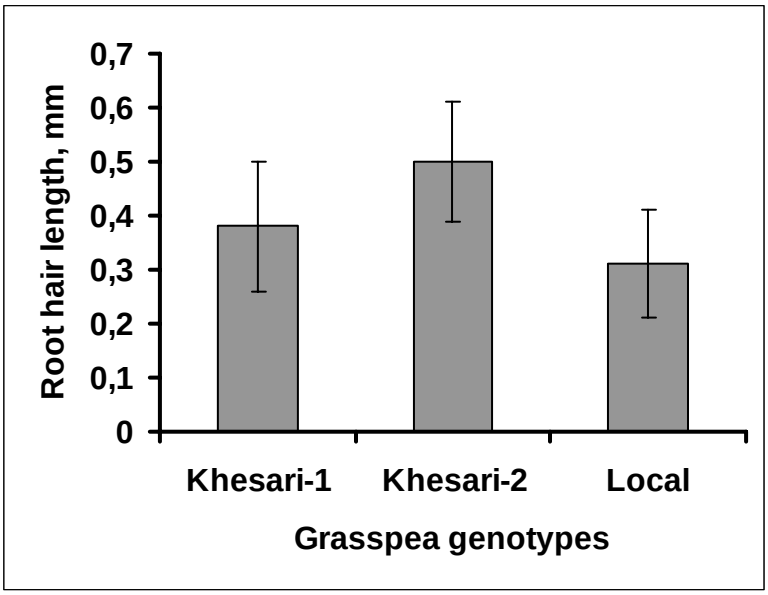

Figure 4

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