

1 Root traits, nutrients uptake, multi-location grain yield and benefit–cost 2 ratio of two lentil (*Lens culinaris*, Medikus.) varieties

3 Tara S. Gahoonia^{1,5}, Omar Ali², A. Sarker³, M. Matiur Rahman⁴ & W. Erskine³

4 ¹The Royal Veterinary and Agricultural University, Plant and Soil Sciences, Thorvaldsensvej 40,

5 DK-1871 Frederiksberg C, Copenhagen, Denmark. ²Pulses Research Center, 6620 Ishurdi, Pabna,

6 Bangladesh. ³International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466,

7 Aleppo, Syria. ⁴Bangladesh Agricultural Research Institute (BARI) Joydebpur, Gazipur 1701, Bangladesh.

8 ⁵Corresponding author*

9 Received ■. Accepted in revised from ■

10 *Key words:* benefit-cost ratio, breeding, genetic diversity, lentil, root system

11 Abstract

12 Lentil is a protein-rich pulse, grown mainly in developing countries as a rain-fed crop in nutrient-poor
13 soils. Hence, the importance of root traits for efficient capture of soil nutrients and water can be crucial
14 to its economical yield. Little is known about the lentil root system and even less about its relationship
15 to grain yield. We compared the root system of two Bangladeshi lentil varieties, Barimasur-3 (BM-3)
16 and Barimasur-4 (BM-4), in a pot experiment and related it to their multi-location grain yield in the
17 fields. BM-4 maintained faster root development both at an early growth stage (20 days after sowing)
18 and at flowering (60 days) compared to BM-3. The roots of BM-4 penetrated the 25 cm depth of the soil
19 profile after 19 ± 1 days and while those of BM-3 took 24 ± 2 days to reach the same depth. The roots
20 of BM-4 were covered with denser ($26 \pm 3 \text{ mm}^{-1}$) and longer (0.48 ± 0.11) root hairs than BM-3 (den-
21 sity $17 \pm 2 \text{ mm}^{-1}$, length 0.32 ± 0.09 mm). The differential presence of root hairs increased the effective
22 length of root system of BM-4 by 12 times and that of BM-3 by five times. The lentil varieties did not
23 differ in their ability to induce pH change and acid phosphatase activity in rhizosphere. In the pot exper-
24 iment, the uptake of macro-nutrients (K, P, Ca, and Mg) as well as micro-nutrients (Fe, Mn, Zn, Cu, B
25 and Mo) by BM-4 was significantly higher, compared to BM-3. The varieties produced the same amount
26 of shoot biomass. At five of six agro-ecological distinct field locations in Bangladesh, BM-4 gave signifi-
27 cantly higher (10–20%) grain yield than BM-3. Linked with the higher grain yield, the benefit-cost ratio
28 (BCR) of BM-4 was 3.14 and that of BM-3 were 2.62, indicating that BM-4 provided better return per
29 unit investment, compared to BM-3, supported by the better root morphology and higher nutrient
30 uptake. This may be one of the reasons supporting the popularity and preferred adoption of BM-4
31 among the Bangladeshi farmers, who grow lentil mainly on nutrient-poor soils. The results indicate the
32 benefits of selection and breeding for superior root traits for better agro-economics.

33

34 Introduction

35 Lentil (*Lens culinaris* L.) is an annual diploid
36 ($2n = 14$), protein-rich staple pulse grown in many
37 developing countries including the Indian

subcontinent, West Asia, North Africa, Sudan, 38
Yemen, Ethiopia, Eritrea and South America; 39
where it complements the cereal-rich diet of the 40
general population, particularly the vegetarians 41
and low-income groups. Approximately half of 42
the world's area cultivated to this crop is estimated 43
to lie in South Asia (Erskine and Saxena, 1993). 44

* Fax No: +45-35283468. E-mail: tsg@kvl.dk

45 In Bangladesh lentils cover about 33% of the
46 total area under pulses and they are, from the
47 consumer's point of view, the most preferred
48 pulse, popularly known as *masur dhal*. Lentil
49 seed is a source of high-quality protein for
50 human and its straw and milling wastes are high
51 value animal feed (Kurdali et al., 1997).

52 Lentil is often grown on nutrient-poor soils
53 with no or little fertilizer applications and is lar-
54 gely a rain-fed crop, often subjected to intermit-
55 tent drought during the growth period and/or
56 terminal drought in the reproductive phase. As
57 receding soil moisture is better conserved in dee-
58 per soil layers, lentil varieties developing larger
59 and deeper root system are advantageous for sus-
60 taining yield in nutrient-poor soils of dry areas.

61 Superior morphological (root length, root
62 hairs) and physiological (exudation of protons
63 and enzymes) root traits facilitate efficient use of
64 existing and added nutrients and water resources
65 in soils and may confer better return of the fertil-
66 izers and irrigation inputs. However, root traits
67 of lentil have rarely been investigated (Sarker
68 et al., 2003) and any linkage of root traits to the
69 economic performance of lentil varieties is largely
70 unknown. The knowledge on the genetic diversity
71 in root traits is desirable, as it will enhance tar-
72 geted breeding of improved varieties, able to
73 resist nutrient and water stresses in the fields of
74 resource-poor growers, ensuring them better har-
75 vests and improved livelihoods.

76 This paper reports the link of various morpho-
77 logical (root length, root hairs), and physiological
78 (rhizosphere pH and exo-cellular phosphatase
79 enzymes) with grain yield and benefit-cost Ratio
80 (BCR) of two lentil varieties.

81 Materials and methods

82 The various root traits of the two lentil varieties,
83 Barimasur-3 (BM-3) and Barimasur-4 (BM-4),
84 were studied, because they are high yielding, but
85 often contrast in grain yield and popularity and
86 adoption among farmers in Bangladesh. Both
87 varieties are small seeded (*microsperma*) and con-
88 sidered to be tolerant/resistance to Rust and
89 *Stemphylium* Blight diseases. The seeds of BM-4
90 are light pink in color, which accords them
91 slightly higher market price. BM-3 was developed
92 through a national hybridization program from a

cross between BLL 79666 (Indian) and local 93
landrace from Pabna Bangladesh (Sarker et al., 94
1999a). BM-4 was developed from the cross 95
between ILL 5888 (improved landrace) and 96
ILL5782 (ICARDA breeding line) at ICARDA- 97
Syria specifically for Bangladesh (Sarker et al., 98
1999b). 99

Soil properties 100

The available data on the properties of soil used 101
in the pot experiment are given below, Soil pH 102
7.7 (0.01 M CaCl₂); organic matter 0.55%; total 103
N 0.029%; major cations extracted with ammo- 104
nium acetate and measured with flame photome- 105
ter (Doll and Lucas, 1973) (meq/100 mL), Ca 106
12.0; Mg 2.5; K 0.25 and other nutrients ($\mu\text{g/g}$) P 107
10.3 (Olsen-P, Olsen et al., 1954); S 20 (Tabata- 108
bai, 1982); B 0.59 (hot water extract, Bingham 109
(1982); Cu 6.3; Fe 11; Mn 6; Zn 1.7 (extracted 110
with DTPA and measured with atomic absorp- 111
tion spectroscopy, Lindsay and Norvell, 1978). 112

Determination of root growth and length 113

The root growth and length of the two varieties 114
was studied in a pot experiment at Pulses 115
Research Center, Ishurdi, Bangladesh. Pots were 116
made by cutting two liter transparent plastic bot- 117
tles (Figure 1a). These were filled with 2.2 kg of 118
soil by shaking to achieve soil bulk density of 119
1.4 g cm⁻³. The soil columns of all the pots were 120
25 cm high. The pots were placed in the open, 121
sides of pots wrapped in black polythene to pre- 122
vent exposure of roots to light and maintained at 123
20% soil moisture by weighing and adding water. 124
Six seeds per pot were planted at 1-cm depth. 125
After germination, three uniform, healthy seed- 126
lings were evenly left in each pot by removing 127
extra seedlings along with the roots. There were 128
four replicates. The date of germination (3- 129
4 days after sowing in both cases) and the date 130
when roots reached the bottom of the pot was 131
recorded to calculate the root penetration time in 132
the soil profile. No major differential disease 133
occurrence was observed in the pot experiment. 134

At 20 and 60 days after sowing, shoots were 135
cut and stored in paper bags for drying. The 136
roots were washed out of soil and cleaned of 137
debris and examined for any differences in nodu- 138
lation. About 1 g of root sample was spread 139

COLOUR FIG.

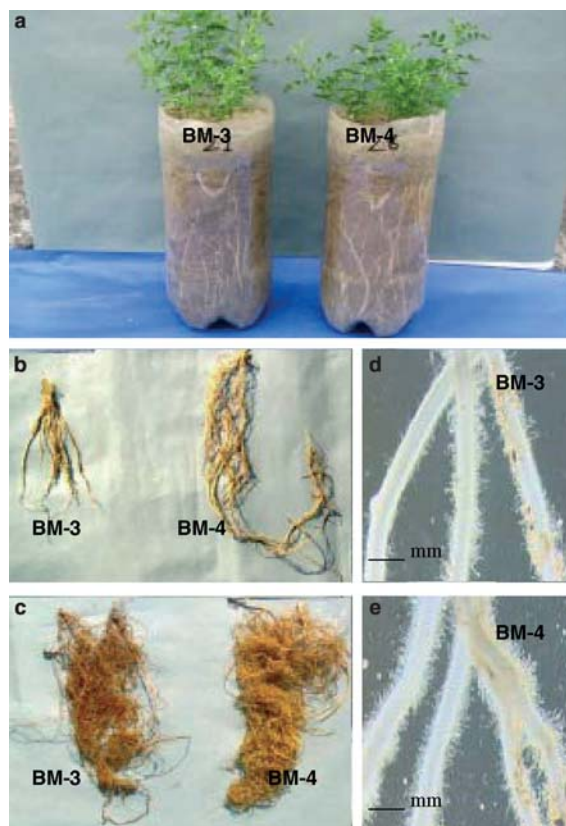


Figure 1. Roots and shoot of two lentil varieties, Barimasur-3 (BM-3) and Barimasur-4 (BM-4). Pots with visible roots and shoot growth of BM-3 and BM-4 at 60 days after sowing (a). Root system of BM-3 and BM-4 at 20 days after sowing (b) and at 60 days after sowing (c). Root hairs of BM-3 (d) and of BM-4 (e).

140 between polythene transparencies and scanned
141 using ScanJet IIcx. The total length of the root
142 system was measured using *Dt-Scan software*
143 (Delta-T Devices, Cambridge, England) as
144 described in Gahoonia et al. (1999).

145 Plant analyses

146 Digestion of plant material

147 Shoots of the pot experiment at flowering stage
148 (60 DAS) was dried at 60 °C until constant weight
149 was recorded. The whole plant material of each
150 pot was ground using a Ultra Centrifugal Mill
151 (Retsch ZM 100). The plant material (0.25 g) was
152 digested in an open vessel system using 70 mL
153 HD polyethylene vials (Capitol Vial Corp, Fulton

Ville, NY, USA) and a graphite-heating block 154
(Mod Block, CPI International, Amsterdam, Hol- 155
land). The plant material was digested at 95 °C 156
using a slight modification of the EPA (Enviro- 157
nmental Protection Agency, USA) Method 3050B, 158
as described below. Five milliliter of 35% HNO₃ 159
(Instra analyzed, Baker, Deventer, Holland) was 160
added to the samples and the samples were boiled 161
for approximately 15 min. After cooling, 2.5 mL 162
70% HNO₃ was added and the samples were 163
reheated. Twenty-five minutes later samples was 164
cooled and 1.5 mL H₂O₂ (Extra pure, Riedel-de- 165
Haën, Seelze, Germany) was applied. When the 166
peroxide reaction ceased, 1 mL of H₂O₂ was 167
added and samples were reheated for approxi- 168
mately 40 min. During the digestion, vials were 169
covered by watch glasses. Samples were cooled 170
overnight and diluted to 50 mL with ultra pure 171
water. For each digestion five blank samples were 172
included. Furthermore samples of a certified refer- 173
ence material-CRM (Apple leaf, standard refer- 174
ence material 1515; National Institute of 175
Standards and Technology, Gaithersburg, MD, 176
USA) were digested to estimate the accuracy and 177
precision of the analysis. Finally, an in-house bar- 178
ley reference material was included in order to 179
keep a check of element concentrations in each 180
individual run on the ICP-MS. Samples were 181
diluted to the same acid concentration (1.75% 182
HNO₃) as standards and quantification was done 183
by external calibration (P/N 4400 ICP-MS, Multi- 184
elemental calibration standard, CPI-International, 185
Amsterdam, Holland). Dilutions were performed 186
in a class 100 laminar flow bench (KR-170s Bio- 187
wizard, Kojair Tech Oy, Vilppula, Finland). 188

ICP-MS and IR-MS 189

A total of 12 elements (K, P, Ca, Mg, S, Fe, Zn, 190
Mn, Cu, B, Mo, Co) were analyzed by ICP-MS 191
(Agilent 7500c, Agilent Technologies, Manches- 192
ter, England). Nitrogen (N) was not analyzed, 193
because lentil, a legume, can fix and make use of 194
atmospheric N₂ and the uptake of N is less 195
dependent on size of the root system. 196

Determination of root hairs 197

The soil was filled in 10 cm long test tubes (diame- 198
ter 3 cm, soil bulk density 1.4 g cm⁻³ and 20% soil 199
moisture, four replicates). One pre-germinated 200
seed was planted in each tube. After 20 days, the 201

- 202 tubes, after cutting the shoot, were immersed in
 203 water overnight in a dark room to prevent mucilage
 204 formation. All roots were removed carefully
 205 using a kitchen sieve and transferred into an Ultra-
 206 sound water bath (Branson 5200, 120W, 47 kHz).
 207 The ultrasound treatment for about 5–10 min
 208 removed remaining soil particles without damag-
 209 ing the root hairs. The root hairs were quantified
 210 using Quantimet 500+ Image Processing and
 211 Analysis System (Leica) at 10× magnification
 212 (Gahoonia and Nielsen, 1997).
- 213 *Determination of rhizosphere pH*
- 214 The roots of 10-days-old seedlings of the two len-
 215 til varieties were embedded in agar containing
 216 pH indicator dye *Bromocresol purple* and
 217 adjusted to pH 6 (Marschner and Romheld,
 218 1983). The root-induced pH change, revealed by
 219 color change, was recorded after one hour fol-
 220 lowing the agar embedding.
- 221 *Rhizosphere phosphatase activity*
- 222 The ability of the lentil varieties to release acid
 223 phosphatase (Aptase) in the rhizosphere was
 224 determined by the method described in Dinkela-
 225 ker and Marschner (1992), based on enzymatic
 226 hydrolysis of 1-naphtylphosphate (substrate) by
 227 root released Aptase, yielding 1-naphtol, which
 228 forms a red complex with Fast Red TR (dye). The
 229 intact roots of 10-days-old seedlings were sand-
 230 wiced between two ashless filter papers, soaked
 231 in a mixture of the dye and the substrate. If the
 232 roots release variable phosphatase enzymes, their
 233 activity is visible as brownish red color of varia-
 234 ble intensity near the roots, because root released
 235 phosphatase produces a brownish red complex
 236 with the dye Fast Red TR after ca. 60 min.
- 237 *Field experiments*
- 238 Field plot trials were conducted in six districts
 239 (Faridpur, Rajshahi, Meherpur, Jhanaidah, Jes-
 240 sore and Kushtia) of Bangladesh, as lentils are
 241 extensively cultivated in these regions. The exper-
 242 iments were laid out during same season in RCB
 243 design, where each location had three replica-
 244 tions. Plot size was 5 × 5 m. All experiments
 245 were sown in the first week of November and
 246 harvested in first week of March. All plots at all
 locations were treated similarly. At harvest grain
 yield was recorded. Chemical sprayings con-
 trolled minor occurrence of diseases of the two
 varieties at all locations.
- Benefit–cost ratio (BCR)*
- Benefit–cost ratio (BCR = gross return per ha/
 total variable costs per ha, Table 2) of a variety
 indicates the amount of money earned by invest-
 ing a given unit amount of the money. To avoid
 confusions, BCR in this paper should be under-
 stood as it is defined here. Gross return was cal-
 culated as the current market value of grains,
 straw and other milling by-products harvested
 per ha. Total variable cost accounts for those
 costs which were purchased or hired (seeds, soil
 preparation and chemical spraying). No fertilizers
 were applied in the experiments. As it was neces-
 sary to treat both the varieties equal at all field
 locations, the total variable costs were also equal
 (Table 2). Due to the attractive seed color of
 BM-4, its market price is slightly better (20 Tak-
 ka kg⁻¹) compared to BM-3 (19 Takka kg⁻¹) and
 the higher value of by-products of BM-4 is
 mainly due to its higher milling waste which fol-
 lowed its higher grain yield (Table 2). As the pro-
 duction of straw did not differ between the
 varieties and it was valued equal.
- Statistical analyses* were performed with Sta-
 tistical Analysis System (SAS) Institute, (1989)
 and Microsoft Excel software as found appropri-
 ate. Statistical significance of the differences
 between the treatments was analyzed by analysis
 of variance (ANOVA).
- Results**
- Root traits, shoot biomass and nutrient uptake*
- The two lentil varieties Barimasur-3 (BM-3) and
 Barimasur-4 (BM-4) differed markedly in root
 length (RL) at early (20 days after sowing, Fig-
 ure 1b) as well as at later (60 days after sowing,
 Figure 1c) reproductive stages. At the early
 growth stage, RL of lentil variety BM-4
 (RL = 4.8 ± 0.15 m per plant) was three times
 larger than that of BM-3 (Figure 2). At 60 days
 after sowing, when the both varieties were flower-
 ing, the RL of BM-4 was 24 ± 1 m plant⁻¹ as

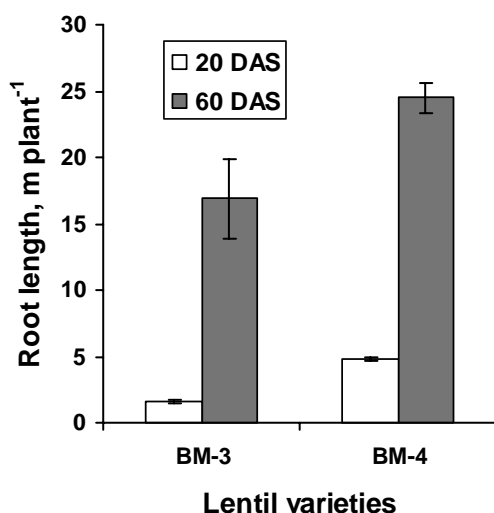


Figure 2. Root lengths of two lentil varieties Barimasur-3 (BM-3) and Barimasur-4 (BM-4), 20 days (20 DAS) and 60 days (60 DAS) after sowing. Bars are standard error of means ($n = 4$).

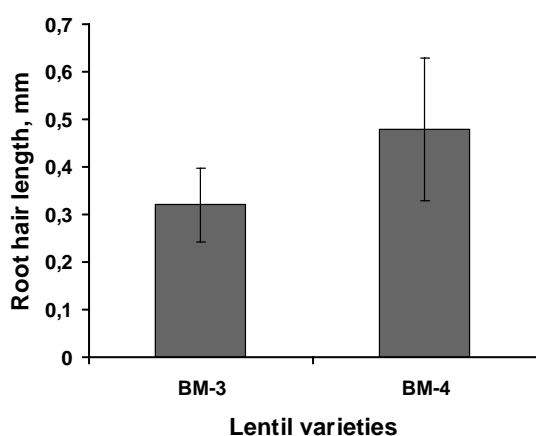


Figure 3. Average root hair lengths of Barimasur-3 (BM-3) and Barimasur-4 (BM-4), 20 days after sowing. Bars are standard error of means ($n = 60$).

292 compared to 17 ± 2 m plant⁻¹ for BM-3 (Fig-
293 ure 2). The roots of BM-4 penetrated the 25 cm
294 depth of the soil profile in 19 ± 1 and that of BM-
295 3 in 24 ± 2 days. The differences between RL at
296 both the growth stages and root penetration of the
297 two varieties were significant ($P < 0.05$).

298 The roots of BM-3 (Figure 1d) were covered
299 with less root hair than BM-4 (Figure 1e). The
300 average root hair length (RHL) of BM-4 was
301 0.48 ± 0.11 mm and that of BM-3 was
302 0.32 ± 0.09 mm (Figure 3). The average root

Table 1. Shoot dry weight (DM) and the nutrients uptake of two lentil varieties Barimasur-3 (BM-3) and Barimasur-4 (BM-4) in the pot experiment. (Mean \pm standard error of means $n = 4$).

DM and nutrients uptake	Variety	
	BM-3	BM-4
DM (g plant ⁻¹)	1.16 ± 0.05	1.12 ± 0.03
<i>Macro-nutrients (g kg⁻¹ DM)</i>		
K	20.43 ± 0.75	28.12 ± 1.03
P	3.62 ± 0.05	3.95 ± 0.06
Ca	6.44 ± 0.04	20.14 ± 0.57
Mg	2.52 ± 0.06	3.06 ± 0.10
S	3.04 ± 0.11	3.05 ± 0.20
<i>Micro-nutrients (mg kg⁻¹ DM)</i>		
Fe	376.2 ± 5.0	400.3 ± 7.0
Mn	49.1 ± 0.21	57.4 ± 1.76
Zn	25.7 ± 1.75	35.18 ± 1.46
Cu	15.6 ± 0.47	20.2 ± 1.52
B	14.7 ± 0.07	16.0 ± 0.72
Mo	1.12 ± 0.02	1.96 ± 0.04
Co	0.26 ± 0.03	0.26 ± 0.02

hair density (RHD, number mm⁻¹ root) of BM-4 303
was 26 ± 3 mm⁻¹ and that of BM-3 was 304
 17 ± 2 mm⁻¹. The differences in RHD between 305
BM-4 and BM-3 were significant ($P < 0.05$), but 306
not in RHL. By using average values of RHL 307
and RHD of the two varieties it was calculated 308
that the differential presence of root hairs 309
increased the effective RL of BM-4 by 12 times 310
and that of BM-3 by five times. The two lentil 311
varieties did not differ in their ability to induce 312
change in rhizosphere pH and rhizosphere 313
Aptase activity (data not shown). 314

The shoot biomass (DM) of the two varieties 315
did not differ ($P < 0.05$) in the pot experiment 316
(Table 1). The uptake of macro-nutrients (K, P, 317
Ca, Mg) as well as micro-nutrients (Fe, Mn, Zn, 318
Cu, B, Mo) by BM-4 was higher compared to 319
BM-3 and the differences were significant ($P < 320$
0.05), except in case of S and Co uptake (Table 1). 321

Field experiments and Benefit-cost ratio (BCR) 322

From the results of field experiments, it appears 323
that BM-4 possesses the ability to translate 324
the advantage of capturing extra nutrients to 325
produce extra grain yield. At five field locations 326
in Bangladesh, BM-4 produced significantly 327
($P < 0.05$) higher grain yield than BM-3 and at 328

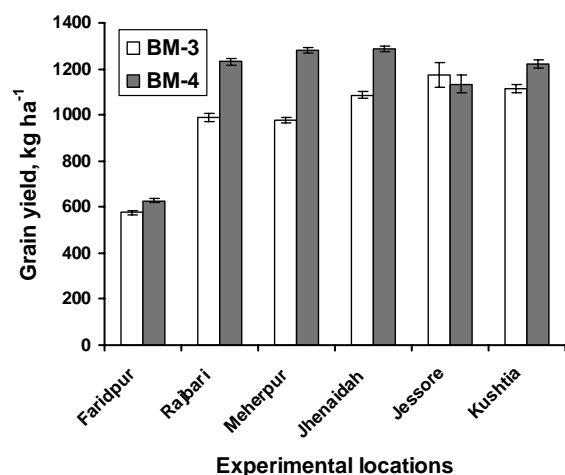


Figure 4. Grain yield of two lentil varieties Barimasur-3 (BM-3) and Barimasur-4 (BM-4) at six locations in Bangladesh. Bars are standard error of means ($n = 3$).

Table 2. Average grain yield of six locations, gross return, total variable cost and benefit cost ratio (BCR) of two lentil varieties Barimasur-3 (BM-3) and Barimasur-4 (BM-4). All money values are given in Bangladesh Takka (Tk).

Items	Lentil varieties	
	BM-3	BM-4
Average grain yield (kg ha ⁻¹) ^a	986	1130
Value of grain yield (Tk ha ⁻¹) ^b	18734	22600
Value of by product (Tk ha ⁻¹) ^c	655	698
Gross return (Tk ha ⁻¹)	19389	23298
Total variable cost (Tk ha ⁻¹)	7409	7409
Cash cost basis		
BCR	2.62	3.14

^aAverage of six locations.

^bBM-3 = 19 Tk kg⁻¹; BM-4 = 20 Tk kg⁻¹ (due to better seed color).

^cHigher value of BM-4 due to higher milling waste which followed its higher grain yield. The value of straw was equal.

329 one location (Jessore) its yield did not differ sig-
 330 nificantly (large variation between the replicates)
 331 from that of BM-3 (Figure 4). The grain yields
 332 of both the varieties differed with location, rang-
 333 ing from 575 ± 8 to 1176 ± 54 kg ha⁻¹ in case
 334 of BM-3 and ranging from 628 ± 11 to
 335 1287 ± 11 kg ha⁻¹ in case of BM-4, showing
 336 that the overall performance of BM-4 was better
 337 than BM-3. Linked with the higher grain yield
 338 and the associated higher amount of milling
 339 by-product, the BCR of BM-4 was 3.14 and that
 340 of BM-3 were 2.62 (Table 2), indicating that

BM-4 provided better return per unit investment 341
 compared to BM-3. This, at least partially, seems 342
 to support the popularity and preferred adoption 343
 of BM-4 among the Bangladeshi farmers, who 344
 often cannot afford to apply fertilizers to their 345
 nutrients-poor soils. 346

Discussion 347

The simple and cost-effective technique applied 348
 to rank the root systems of the two varieties 349
 allowed visual assessment of the growth and pen- 350
 etration of roots in soil, before actually washing 351
 them out and measuring their length using image 352
 analysis. This systematic approach reduced the 353
 likelihood of the major errors, usually associated 354
 with root measurements, as indicated by the 355
 agreement between the visual observations (Fig- 356
 ure 1) and quantitative measurements (Figure 2). 357
 Limiting the investigation only to the two con- 358
 trasting varieties offered the advantage that 359
 major morphological and physiological root 360
 traits, known to influence the capture of nutrients 361
 and soil moisture, could be studied in detail for 362
 examining their linkage to grain yield in multi- 363
 location field experiments. The study suggested a 364
 strong link between morphological root traits, 365
 nutrients uptake, grain yield and BCR as the 366
 indicators of economic output of the two lentil 367
 varieties. The much lower yields of both the 368
 varieties at the Faridpur location (Figure 4) was due 369
 to heavy rain at the flowering stage, which 370
 affected the crop performance. Even then BM-4 371
 was superior than BM-3. 372

The results of this study suggest that the vari- 373
 ation in root morphology of the two varieties is 374
 pronounced, without the variation in the ability 375
 to induce chemical (rhizosphere pH) and bio- 376
 chemical (Aptase) change in the rhizosphere envi- 377
 ronment through root exudation. Root induced 378
 rhizosphere pH is known to influence availability 379
 of soil inorganic phosphorus (Gahoonia and 380
 Nielsen, 1992) and micro-nutrients to plants 381
 (Marschner and Römheld, 1996). The role of Apt- 382
 ase for catalyzing the conversion of soil organic 383
 phosphorus into plant available inorganic phos- 384
 phorus is also reported (Asmar et al., 1995). The 385
 lack of variation in the rhizosphere pH and 386
 Aptase among the two varieties, two nutrient 387
 mobilizing processes, suggested that root 388

389 morphology traits, enhancing the exploration of
390 soil for nutrients (Table 1) and water, might be a
391 criterion worth giving more attention for the
392 selection of nutrient efficient and drought toler-
393 ant varieties for nutrient limiting and dry soils.
394 This finding is supported by the results of other
395 recent studies where genetic diversity was found
396 in root size of lentil (Sarker et al., 2003) and root
397 hair formation of soybean (Wang et al., 2004),
398 common bean (Yan and Lynch, 1998) and cow-
399 pea (Krasilnikoff et al., 2003).

400 The roots of both varieties were covered with
401 root hairs, but the disparity in the presence of
402 root hairs on the roots extended the effective root
403 length of BM-4 by 12 times as compared that of
404 BM-3 by five times. This enormous extension of
405 the effective root length by root hairs conferred
406 extra advantage for BM-4 to absorb most of the
407 nutrients from the soil (Table 1), also reported in
408 other studies (Bates and Lynch, 2000; Gahoonia
409 and Nielsen 1998; Hofer, 1996). The root hair
410 production on the roots is enhanced as a
411 response to limitation of water and phosphorus
412 (Bates and Lynch, 1996; Gahoonia et al., 1999).
413 Additionally root hair formation is a photosyn-
414 thetic carbon saving strategy for extending the
415 root surface area i.e., three times by investing
416 only 2% of the root weight (Clarkson, 1996;
417 Röhm and Werner, 1987).

418 Bangladeshi soils are generally low in both
419 macro- and micro-nutrients (Yusuf Ali et al.,
420 2002). In pot experiment, BM-4 absorbed signifi-
421 cantly higher amount of nutrients (Table 1) and
422 such ability of BM-4 may have supported to pro-
423 duce higher grain yields in multi-location field tri-
424 als (Figure 4). Lentil is rain-fed winter crop and
425 winter is dry in Bangladesh. Therefore, in addition
426 to higher absorption of soil nutrients, better cap-
427 ture of soil moisture might have played a role in
428 better performance of BM-4, which was not inves-
429 tigated in the present study. Due to very small
430 diameter (5–10 μm), root hairs are able to pene-
431 trate and grow into tiny soil pores to extract water,
432 not directly accessible to roots as such. Hence,
433 varieties with abundant root hairs on their roots
434 can be expected to be superior in using soil water
435 more efficiently, when soil moisture is receding.
436 This together with faster growth of roots into the
437 deeper soil layers may be expected to provide extra
438 advantages for BM-4 in capturing soil nutrients
439 and very likely also soil moisture.

440 Root hair trait followed monogenic Mendel's
441 law of inheritance as it was indicated by 3:1
442 segregation ratio of F_2 generation, when a bold
443 root barley, *brb* mutant and its wild type was
444 crossed (Gahoonia et al., 2001), suggesting that
445 the trait may be easy to handle in breeding.
446 Comparable to the variation in root hair trait
447 of lentil varieties reported here, a wide genetic
448 variation in root hair formation of other grain
449 legumes like common bean (Yan and Lynch,
450 1998), soybean (Wang et al., 2004) and cowpea
451 (Krasilnikoff et al., 2003) has been reported.
452 QTL mapping of root hair trait is progressing
453 (Yan et al., 2004).

454 In the pot experiment, no inoculation was
455 applied and both the varieties formed nodules,
456 but they did not show differences in nodulation
457 at the time of flowering (60 days after sowing),
458 when the roots were washed out and examined
459 visually for the presence of nodules. In the field
460 experiments, no inoculation was applied and
461 the nodulation ability of the varieties was not
462 determined and the link of potential nitrogen
463 fixation ability to the differential grain yield
464 could not be determined. Other studies (Shah
465 et al., 2000) suggest that inoculation has signifi-
466 cant benefits for nodulation, biomass, grain
467 yield, nitrogen and phosphorus uptake, irrespec-
468 tive of the levels of nitrogen and phosphorus
469 in soil.

470 The higher potential return of investment in
471 the cultivation of lentil variety BM-4, as indicated
472 by the higher BCR values (Table 2) which was
473 related to its better root system and better nutri-
474 ents uptake might be one of the reasons support-
475 ing its higher yield and popularity among the local
476 farmers. However, it must be kept in mind that a
477 number other factors, like the cooking quality,
478 seed size and color, the availability of seeds and
479 resistance to diseases may also affect the popular-
480 ity and adoption of the varieties. The results of
481 this study indicate the economical utility of explor-
482 ing the genetic diversity in root traits of lentil
483 genotypes/landraces. The superior root traits can
484 then be incorporated in disease resistant and other
485 superior agronomic backgrounds for breeding of
486 high and stable yielding varieties. Hamdi (1992)
487 reported high broad-sense heritability (65-85%) of
488 root morphological traits in lentil, signifying the
489 feasibility of using them successfully in the breed-
490 ing programs.

491 Acknowledgments

492 The financial support of Danish Council for
493 Development Research (RUF), Copenhagen, for
494 conducting the research, is thankfully acknowl-
495 edged. Thanks to scientific assistant *Md. Saiful*
496 *Islam Khan* for helping with the experimental
497 work at Pulses Research Center, Ishurdi, Bangla-
498 desh. Thanks to Adrian Bolliger for reading and
499 improving the manuscript.

500 References

- 501 Asmar F, Gahoonia T S and Nielsen N E 1995 Barley geno-
502 types differ in extracellular phosphatase activity and deple-
503 tion of organic phosphorus from rhizosphere. *Plant Soil*
504 172, 117–122.
- 505 Bates T R and Lynch J P 1996 Stimulation of root hair elon-
506 gation in *Arabidopsis thaliana* by low phosphorus availabil-
507 ity. *Plant Cell Environ.* 19, 529–538.
- 508 Bates T R and Lynch J P 2000 The efficiency of *Arabidopsis*
509 *thaliana* (Brassicaceae) root hairs in phosphorus acquisi-
510 tion. *Am. J. Bot.* 87, 964–970.
- 511 Bingham F T 1982 Boron. *In Methods of Soil Analysis.*
512 Part 2. Eds. A L Page, R H Miller and Keeney, p. 431.
513 American Society of Agronomy Inc. Madison, Wisconsin
514 USA.
- 515 Clarkson D T 1996 Root structure and sites of ion uptake.
516 *In Plant Roots: The Hidden Half.* Marcel Dekker Eds. Y.
517 Waisel A, Eshel U, Kafkaki. pp. 483–510. Inc. New York,
518 Basel, Hongkong.
- 519 Dinkelaker B and Marschner H 1992 *In vivo* demonstration
520 of acid phosphatase activity in the rhizosphere of soil
521 grown plants. *Plant Soil* 144, 199–205.
- 522 Doll E C and Lucas R E 1973 Testing soil for potassium, cal-
523 cium and magnesium. *In Soil Testing and Plant Analysis.*
524 Eds. L M Walsh and J D Beaton. pp. 133–152. Soil Sci-
525 ence Society of Am. Madison, Wisconsin, USA.
- 526 Erskine W and Saxena M C (Eds.) 1993 Lentil in South Asia.
527 Proceedings of the Seminar on Lentils in South Asia, 11–
528 15 March 1991, New Delhi, India. ICARDA, Aleppo,
529 Syria. vi + 236 pp. ISBN 92-9127-003-2.
- 530 Gahoonia T S and Nielsen N E 1992 The effect of root
531 induced pH changes on the depletion of inorganic and
532 organic phosphorus in the rhizosphere. *Plant Soil* 143,
533 185–189.
- 534 Gahoonia T S and Nielsen N E 1997 Variation in root hairs
535 of barley cultivars doubled phosphorus uptake from soil.
536 *Euphytica* 98, 177–182.
- 537 Gahoonia T S and Nielsen N E 1998 Direct evidence on par-
538 ticipation of root hairs in phosphorus (³²P) uptake from
539 soil. *Plant Soil* 198, 147–152.
- 540 Gahoonia T S, Nielsen N E and Lyshede O B 1999 Phospho-
541 rus (P) acquisition of cereal cultivars in the field of three
542 levels P fertilization. *Plant Soil* 211, 269–281.
- 543 Gahoonia T S, Nielsen N E, Joshi P A and Jahoor A 2001 A
544 root hairless barley mutant for elucidating genetics of root
545 hairs and phosphorus uptake. *Plant Soil* 235, 211–219.
- Hamdi A 1992 Heritability and combining ability of root
characters in lentil (*Lens culinaris* Medikus). *Egyptian*
J. Agric. Res. 70, 247–255.
- Hofer R M 1996 Root hairs. *In Plant Roots: The Hidden*
Half. Eds. Y. Waisel, A Eshel, U Kafkai. pp. 111–126.
Marcel Dekker, Inc. New York, Basel, Hongkong.
- Krasilinikoff G, Gahoonia T S and Nielsen N E 2003 Vari-
ation in phosphorus uptake of cowpea genotypes (*Vigna*
unguiculata) due to differences in root and root hair
length and root-induced rhizosphere processes. *Plant Soil*
251, 83–91.
- Kurdali F, Kalifo K and Al Schamma M 1997 Cultivar
differences in nitrogen assimilation, partitioning and
mobilization in rain-fed grown lentil. *Field Crop. Res.*
54, 235–243.
- Lindsay W L and Norvell W A 1978 Development of DTPA
soil test for zinc, iron, manganese, and copper. *Soil Sci.*
Soc. Am. J. 42, 421–428.
- Marschner H and Römheld V 1983 *In vivo* measurements of
root induced pH change at soil–root interface. Effect of
plant species and nitrogen source. *Z. Pflanzenphysiol.* 111,
241–251
- Marschner H and Römheld V 1996 Root induced changes in
the availability of micronutrients in the rhizosphere. *In*
Plant Roots: The Hidden Half. Eds. Y Waisel, A. Eshel
and U kafkaki. pp. 557–580. Marcel Dekker, Inc. New
York, basel, Hong kong.
- Olsen S R, Cole C V, Watanabe F S and Dean L A 1954
Estimation of Available Phosphorus in Soils by Extraction
with Sodium Bicarbonate. USDA Circ. 939. U S Print
Office, Washington DC.
- Röhm M and Werner D 1987 Isolation of root hairs from
seedlings of *Pisum sativum*. Identification of root hair spe-
cific proteins by *in situ* labelling. *Physiol. Plant.* 69, 129–
136.
- Sarker A, Kumar J, Rahman M M, Hassan M S, Zaman W,
Afzal M A and Murshed A N M M 1999a Registration of
“Barimasur-3” Lentil. *Crop Sci.* 39, 1536.
- Sarker A, Erskine W, Hassan M S, Afzal M A and Murshed
A N M M 1999b Registration of “Barimasur-4” Lentil.
Crop Sci. 39, 876.
- Sarker A, Erskine W and Singh M 2003 Variation in shoot
and root characteristics and their association with drought
tolerance in lentil landraces. *Genet. Resour. Crop Evol.* (in
press).
- SAS Institute Inc. 1989 SAS/STAT. User Guide. Version 5.
SAS Institute Inc., Cary, NC, USA.
- Shah N H, Hafeez F Y, Arshad M and Malik K A 2000
Response of lentil to Rhizobium leguminosarum bv viciae
strains at different levels of nitrogen and phosphorus.
Austr. J. Exp. Agri. 40, 93–98.
- Tabatabai M A 1982 Sulfur *In Methods of Soil Analysis.* Part
2. Eds. A L Page, R H Miller and Keeney, p. 501. Ameri-
can Society of Agronomy Inc. Madison, Wisconsin, USA.
- Wang L, Liao H, Yan X, Zhuang B and Dong Y 2004
Genetic variability for root hair traits as related to phos-
phorus status in soybean. *Plant Soil* (in press)
- Yan X and Lynch J P 1998 Genetic variation for root hair den-
sity and length in common bean in response to low
phosphorus availability. *In Phosphorus in Plant Biology:
Regulatory Roles in Molecular, Cellular, organisms and
Ecosystem Processes.* Eds. J P Lynch and J. Deikman.
American Society of Plant Physiologist, Vol.19, Rockville,
Maryland, USA.



Journal : **PLSO** SPS Article No. : **DO00004573**
PIPS No. : **DO00004573**
MS Code : **PLSO836R2**

Dispatch : **28-10-2004**

Pages : **9**

LE
 CP

TYPESET
 DISK

- 610 Yan X, Hong Liao H, Beebe S E, Matthew W, Blair M W
 611 and Lynch J P 2004 QTL mapping of root hair and acid
 612 exudation traits and their relationship to phosphorus
 613 uptake in common bean. Plant Soil (in press)
 614 Yusuf Ali M, Krishnamurthy L, Saxena N P, Rupela O P,
 615 Kumar J and Johansen C 2002 Scope for genetic manipula-


tion of mineral acquisition in chickpea. Plant and Soil 245,
 123-134.

616
 617

Section editor: ■

618

UNCORRECTED PROOF

	Journal : PLSO SPS Article No. : DO00004573	Dispatch : 28-10-2004	Pages : 9
	PIPS No. : DO00004573	<input type="checkbox"/> LE	<input type="checkbox"/> TYPESET
	MS Code : PLSO836R2	<input checked="" type="checkbox"/> CP	<input checked="" type="checkbox"/> DISK