Genetic diversity of seed storage protein in the Ethiopian garden cress (Lepidium sativum L.)

Legesse Tadesse¹,², Firew Mekbib², Adugna Wakjira³ and Zerihun Tadele⁴*

¹Department of Biology, College of Natural and Computational Sciences, Dire Dawa University, P. O. Box 1362, Dire Dawa, Ethiopia.
²College of Agriculture and Environmental Sciences, School of Plant Sciences, Haramaya University, P. O. Box 138, Dire Dawa, Ethiopia.
³Ethiopian Institute of Agricultural Research, P. O. Box 2003, Addis Ababa, Ethiopia.
⁴Institute of Plant Sciences, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland.

Received 6 July 2018; Accepted 3 September, 2018

The Ethiopian garden cress (Lepidium sativum L.) is an important crop extensively used as food and medicine. In this study, total seed storage proteins of 112 garden cress genotypes collected from diverse growing regions in Ethiopia were investigated to assess patterns of genetic diversity and relationships. Using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), a total of 1774 stable protein bands were identified through discontinuous electrophoresis. Of these, 1597 bands were polymorphic. A maximum of 20 protein sub-units in the range of 15 to 75 kDa were observed per genotype. The similarity coefficient among these genotypes ranged from 0.25 to 1.00 with an average genetic dissimilarity of 0.2754. On the basis of Ward Euclidian distance, the genotypes were grouped into five major clusters, the largest one comprised of 62 genotypes (Cluster III) followed by 26 genotypes in Cluster I. Cluster IV and V contained a total of 14 genotypes that were the most distantly related to other groups, and thus can be potentially used as parents for exploitation of heterotic effects in hybrid breeding programs. Our findings using SDS-PAGE profiles revealed no obvious association between geographic region of origin and germplasm clustering. However, the polymorphism and cluster analysis indicated that garden cress genotypes differed greatly in the composition of seed proteins. This shows that protein profiling could be used as a rapid and reliable method for genetic diversity studies. In order to fully explore the protein based genetic diversity in garden cress germplasm, techniques such as 2-D gel electrophoresis are recommended in future studies.

Key words: Cluster analysis, dissimilarity index, garden cress, Lepidium sativum, protein polymorphism, protein profiling, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), seed storage protein.

INTRODUCTION

Garden cress (Lepidium sativum L.) belongs to the family Brassicaceae and the genus Lepidium which contains about 150 species that are distributed throughout almost all temperate and subtropical regions of the world (Bermejo and Leon, 1994; Wadhwa et al., 2012; Rava, 2016). Garden cress is widely cultivated in Africa, Europe, Russia and North America. It is a fast growing, edible herb and an important medicinal plant having a wide range of desirable effects on human health since the Vedic era about 3000 years ago (Manohar et al.,...
Various beneficial effects have been observed with the consumption of garden cress. Different parts of the plant including roots, seeds and leaves have been used as a source of functional food and/or medicine (Rava, 2016). The seeds and leaves of garden cress contain volatile oils and have been consumed as salad and as spice (Amare, 1976; Wadhwa et al., 2012; Rava, 2016). Its seeds are rich source of proteins, carbohydrate, fat (for example omega-3 fatty acids), dietary fiber, vitamins (tocopherol, β-carotene and ascorbic acids), minerals (K, Mg, P, Ca, Fe), and other essential nutrients and phytochemicals (Gokavi et al., 2004; Doke and Guha, 2014). This indicates that the seeds of garden cress play vital role as a promising multipurpose medicinal and nutritional plant. Nowadays, garden cress is becoming popular not only because of its superior medicinal and nutritional values but also due to its contribution in the biofortification of nutritionally inferior crops (Manohar et al., 2012; Singh et al., 2015) in order to ensure the nutritional security of the global population.

Diversity existing in germplasms of crops collected from diverse growing regions need to be properly characterized and evaluated to improve strategies for conservation and utilization towards cultivar development (Parashar et al., 2015; Sharma and Krishna, 2017). Several strategies have been adopted for germplasm characterization including the use of morphological markers (Kancherla and Bhalla, 2003), seed storage protein markers and DNA molecular markers (Rahman and Hirata, 2004). Using quantitative, biochemical and molecular markers suitable germplasm for future plant breeding programs can be identified. Studies in 49 Ethiopian garden cress landraces using morphological and yield-related traits revealed huge variability at diverse agro-ecological zones (Temesgen et al., 2013a, b). Similarly, large diversity was recorded among 85 Ethiopian garden cress genotypes using inter simple sequence repeat (ISSR) (Said and Kassahun, 2015).

Storage or structural seed proteins, encoded by families of polymorphic genes (Mandal and Mandal, 2000) have been extensively used as a genetic marker as they are largely independent of environmental fluctuations (Hameed et al., 2009). Unlike morphological markers (Siddiqui and Naz, 2009), the banding patterns of protein markers are stable (Iqbal et al., 2005; Nasar et al., 2006; Iqbal et al., 2014). Due to these benefits, protein markers have been widely applied in the analysis of genetic diversity within and between accessions, in studying plant domestication in relation to genetic resource conservation and breeding, and in establishing genome relationships (Kakaei and Kahrizi, 2011; Hameed et al., 2012; Sharma and Krishna, 2017). Seed storage protein profiling has been used in investigating diversity among selected varieties of Brassica napus (Nasar et al., 2006; Choudhary et al., 2015). Similarly, seed protein profiles were instrumental in the identification of intra-specific genetic divergence in rape seed (Khan et al., 2014).

The use of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in studying plant proteins was found to be simple and inexpensive; hence, it has applications in the improvement of the crop of interest through breeding (Zada et al., 2013; Sharma and Krishna, 2017). However, so far, only few studies were made regarding the diversity of storage proteins in the garden cress germplasm. Gianazza et al. (2007) examined the influence of different concentrations of cadmium on the garden cress plants using seed protein storage marker. However, genetic diversity in the seed protein of garden cress was so far not made using the SDS-PAGE, at least in the Ethiopian germplasm collections. Hence, the current study was conducted to investigate using SDS-PAGE diversity in the seed storage protein of the Ethiopian garden cress accessions collected from diverse agro-ecological regions in the country.

**MATERIALS AND METHODS**

**Plant materials and protein samples**

Lists of plant materials used in the study and their origin are presented in Table 1. For extraction of proteins, mortar and pestle were used to crush and grind seeds of each genotype. Defatting of about 0.1 g of flour was carried out with chloroform, methanol and acetone in the ratio of 2:1:1 as described by Geetha and Balamurugan (2011) for mustard genotypes. Next day, about 0.05 g of flour was suspended in 1 ml extraction buffer (0.125 M Tris-Cl, 4% SDS, 20% v/v glycerol, 1% 0.2 M DTT, 25 mM EDTA, pH 6.8). Homogenized mixture was incubated at room temperature for 3 h.

Protein extraction buffer was properly mixed by vortexing for 5 to 10 min intermittently. The solubilized samples were centrifuged at 14000 rpm for 10 min at 4°C, and the clear supernatant saved at 4°C until they were run gel electrophoresis following the methods of Roy and Kumar (2014) and Buckseth and Singh (2016) with some modification. The gel electrophoresis was run by mixing of 30 µl of the protein extract with 30 µl of sample loading buffer (0.1% of bromophenol blue, 2% SDS, 6% v/v glycerol, 2.5% 0.5 M Tris-HCL PH 6.8 and 1% 0.5 M DTT) followed by vortexing and heating at 65°C for 10 min to ensure complete denaturation, and then with brief vortexing and spinning at room temperature just before loading on gel (Bollag et al., 2002; Nisar et al., 2016).

The SDS-PAGE was carried out in various combinations and optimized to 12.5% acrylamide gel concentration and by loading 30 µl of samples to obtain the best resolution. The procedure
| Accession ID | Regional state | Zone       | District/location       |
|-------------|----------------|------------|-------------------------|
| 229799      | Amhara         | East Gojam | Enbise SarMidir         |
| 229798      | Amhara         | East Gojam | Hulet Ej Enese          |
| CG14        | Amhara         | North Gojam| Goncha Siso Enese       |
| 235892      | Amhara         | North Gondar| Addi Arkay             |
| 214243      | Amhara         | North Gondar| Debark                  |
| 205163      | Amhara         | North Gondar| Debark                  |
| 205162      | Amhara         | North Gondar| Debark                  |
| 208030      | Amhara         | North Gondar| Gondar Zuria           |
| CG12        | Amhara         | North Shewa| Efratana Gidim         |
| 229203      | Amhara         | North Shewa| Lay BetnaTach Bet       |
| 229202      | Amhara         | North Shewa| Lay BetnaTach Bet       |
| 229200      | Amhara         | North Shewa| Lay BetnaTach Bet       |
| 229201      | Amhara         | North Shewa| Lay BetnaTach Bet       |
| 229204      | Amhara         | North Shewa| Lay BetnaTach Bet       |
| 229199      | Amhara         | North Shewa| Siyadebrina Wayu Ens    |
| 229205      | Amhara         | North Shewa| Weremo Wajetuna Mid     |
| 241777      | Amhara         | North Wello| Guba Lafto              |
| 207542      | Amhara         | South Gondar| Kemekem                 |
| 90004       | Amhara         | South Gondar| Tach Gayint            |
| 90018       | Amhara         | South Wello| Debresina               |
| CG7         | Amhara         | South Wello| Debresina               |
| 90020       | Amhara         | South Wello| Dessie Zuria            |
| 212628      | Amhara         | South Wello| Kutaber                 |
| 215714      | Amhara         | South Wello| Werebabu                |
| 215713      | Amhara         | South Wello| Werebabu                |
| CG11        | Amhara         | South Wello| Werebabu                |
| CG22        | Amhara         | South Wello| Werebabu                |
| 90012       | Unknown        |            |                         |
| 233679      | Unknown        |            |                         |
| 233370      | Unknown        |            |                         |
| 240579      | Unknown        |            |                         |
| 90010       | Unknown        |            |                         |
| 90009       | Unknown        |            |                         |
| 90014       | Unknown        |            |                         |
| 90017       | Unknown        |            |                         |
| 90007       | Unknown        |            |                         |
| 90008       | Unknown        |            |                         |
| 205141      | SNNP           | Gurage     | Goro                    |
| 242916      | SNNP           | Keficho Shekicho| Chena          |
| 240396      | SNNP           | Keficho Shekicho| Decha      |
| 240397      | SNNP           | Keficho Shekicho| Decha      |
| 202116      | SNNP           | Keficho Shekicho| Ginbo      |
| CG17        | SNNP           | North Omo  | Basketo                 |
| 225725      | SNNP           | North Omo  | Bonke                   |
| CG13        | SNNP           | North Omo  | Damot Wayde             |
| 8604        | SNNP           | North Omo  | Damot Weyde             |
| 240808      | SNNP           | North Omo  | Damot Weyde             |
| 90016       | SNNP           | North Omo  | Gofa Zuria              |
| CG16        | SNNP           | North Omo  | Gofa Zuria              |
| Code  | Region   | Zone           | Town                  |
|-------|----------|----------------|-----------------------|
| 225799| SNNP     | North Omo      | Kemba                 |
| CG18  | SNNP     | North Omo      | Mareka Gena           |
| 240578| SNNP     | North Omo      | Melokoza              |
| CG21  | Tigray   | West Tigray    | Shire                 |
| 233985| Tigray   | West Tigray    | Laelay Adiyabo        |
| 219959| Tigray   | West Tigray    | Medebeay Zana         |
| 216885| Oromia   | Arssi          | Merti                 |
| 216886| Oromia   | Arssi          | Merti                 |
| CG15  | Oromia   | Arssi          | Tiyo                  |
| CG20  | Oromia   | Bale           | Melyu                 |
| 237991| Oromia   | Bale           | Adaba                 |
| CG4   | Oromia   | Bale           | Agarfa                |
| CG5   | Oromia   | Bale           | Dinsho                |
| CG9   | Oromia   | Bale           | Gaserana Gololcha     |
| 19001 | Oromia   | Bale           | Ginir                 |
| 212852| Oromia   | Bale           | Goro                  |
| 19002 | Oromia   | Bale           | Goro                  |
| 212853| Oromia   | Bale           | Goro                  |
| 90002 | Oromia   | Bale           | Sinanana Dinscho      |
| CG6   | Oromia   | Bale           | Sinanana Dinscho      |
| 230524| Oromia   | Bale           | Girawa                |
| CG10  | Oromia   | East Shewa     | Akaki                 |
| 90006 | Oromia   | East Hararghe  | Deder                 |
| 90005 | Oromia   | East Hararghe  | Deder                 |
| 230831| Oromia   | East Hararghe  | Girawa                |
| 208693| Oromia   | East Hararghe  | Gursum                |
| 216816| Oromia   | East Hararghe  | Gursum                |
| 230830| Oromia   | East Hararghe  | Jarso                 |
| 208669| Oromia   | East Hararghe  | Kersa                 |
| 234828| Oromia   | East Wellega   | Diga Leka             |
| CG8   | Oromia   | Jimma          | Limu Seka             |
| 18843 | Oromia   | North Shewa    | Debre Libanos         |
| 208666| Oromia   | West Hararghe  | Mieso                 |
| 18841 | Oromia   | West Shewa     | Bako                  |
| 90021 | Oromia   | West Shewa     | Cheliya               |
| 19000 | Oromia   | West Hararghe  | Chiro                 |
| CG2   | Oromia   | West Hararghe  | Chiro/Wachu           |
| CG3   | Oromia   | West Hararghe  | Gemechis              |
| 208667| Oromia   | West Hararghe  | Habro                 |
| CG19  | Oromia   | West Hararghe  | Mesela                |
| CG1   | Oromia   | West Shewa     | Wolmera               |
| 90022 | Oromia   | West Wellega   | Dale Lalo             |
| 208769| Oromia   | West Wellega   | Sayo                  |
| 215808| Oromia   | West Wellega   | Sayo                  |
| 215807| Oromia   | West Wellega   | Sayo                  |
| 230829| Somalia  | Jigjiga        |                       |
| 230523| Somalia  | Jigjiga        |                       |
| 216815| Somalia  | Jigjiga        |                       |
| 231210| Somalia  | Jigjiga        |                       |
| 233982| Tigray   | Central Tigray | Adwa                  |
| 237512| Tigray   | Central Tigray | Adwa                  |
developed by Laemmli (1970) was followed for gel preparation and running. 30 µl of samples digested was loaded into the wells of 4% acrylamide stacking gel (1.5 mm thick) for protein separation. Electrophoresis was carried out at a constant 250 V for the medium slab vertical gel apparatus until bromophenol blue marker crossed bottom of the gel. Pre-stained protein marker, ranging from 10 to 250 kDa (precision plus protein dual color standard supplied by BIO-RAD) was run for reference to molecular weight of respective protein bands in kDa. After complete run, the gels were fixed and stained with 0.5% coomassie brilliant blue (CBB) R-250 in acetic acid: methanol: water (10:40:50 volume ratio) for 3 h and destained in the same acetic acid-methanol-water solution except CBB for overnight (Bollag et al., 2002; Sadia et al., 2009) with constant and gentle shaking.

Data analysis

Gel evaluation for data scoring was done on a light box and rechecked by using photograph that was taken by high resolution camera supported by white light illuminator. The experiment was repeated twice to check the reproducibility of the protein bands. A band presence was coded (1), while the absence of bands scored as (0). Only reproducible bands occurring in high frequency were scored by identifying each protein band carried out according to standard proteins. The intensity of bands was not taken into consideration but only the presence of the bands was taken as indicative. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, similarity index was calculated for all possible pairs of protein type’s electrophore-grams. Similarities among genotypes were estimated using Jaccard coefficient of similarity (Jaccard, 1908). Depending on the electrophoretic band spectra, similarity index (S) was designed for all pairs of protein band pattern by the subsequent formula: S = a/ (a + b) where S = similarity index, a = Number of bands common to a and b protein types, b = Number of bands in protein type ‘b’. The similarity matrix was generated and converted to a dissimilarity matrix. The different bands in the range of 15-75 kDa were used for calculation of similarity indices. Polymorphism % was calculated using the formula:

Polymorphism (%) = [Number of polymorphic bands/Total number of bands] x 100.

The generated data matrix was then used for descriptive statistics and for constructing dendrogram by the ward method using Darwin version 6 Software.

---

**Table 1. Contd.**

| Sample No. | Region | Sub Region | Genotype |
|------------|--------|------------|----------|
| 219961     | Tigray | Central Tigray | Adwa      |
| 207910     | Tigray | Central Tigray | Adwa      |
| 219958     | Tigray | Central Tigray | Laelay Maychew |
| 233984     | Tigray | Central Tigray | Werielehe |
| 233983     | Tigray | Central Tigray | Werielehe |
| 238273     | Tigray | Central Tigray | Adwa      |
| 219962     | Tigray | Central Tigray | Nae Der Ainet |
| 233981     | Tigray | East Tigray | Ganta Afeshum |
| 242609     | Tigray | East Tigray | Ganta Afeshum |
| 234355     | Tigray | East Tigray | Ganta Afeshum |

SNNP: Southern Nations and Nationality of People Regional State.

---

**RESULTS AND DISCUSSION**

**Genetic diversity in seed storage protein**

A typical electrophoretic banding patterns and their distribution is presented in Figure 1. A maximum of 20 protein sub-units were observed per genotype within the range of protein molecular weight of 15 to 75 kDa. A total of 1774 polypeptide bands with an average of 15.8 bands per genotype were obtained (Table 2). Out of the total of 1774 bands, 1597 were polymorphic while 177 were monomorphic (data not shown). The banding patterns revealed large variations among genotypes in the low molecular weight protein profiles. This revealed considerable variations in five regions (A to E) (Figure 1). Region A contains relatively high molecular weight proteins ranging from 50 to 75 kDa while in Region B, three protein sub-units ranging in size from 37 to 50 kDa are observed. Five protein bands were found for Region C which ranged from 25 to 37 kDa while Region D comprised of four protein sub units ranging from 20 to 25 kDa. The last part, Region E, contains small size proteins with molecular weight ranging from 15 to 20 kDa. All these five regions showed both light and dark stained bands and were polymorphic except for the last two bands of Region E which were monomorphic (Table 1). This indicates that the proportion of polymorphic bands over the total bands detected were 90%. Ten genotypes (namely, 241777, 229203, 235892, 229205, 216816, 214243, 208030, 229199, 237991, 216816 and CG2) showed the highest number of protein bands (each 20 bands) followed by 12 genotypes (207542, 229202, 229799, 216816, 229204, CG14, 238273, 90021, 208667, 219961, 242609, and CG6) each with 19 bands. Three genotypes (90002, 229799 and 90022) had minimum number of protein bands ranging from 7 to 9. Protein sub units located on band number 19 and 20 were the most frequent (Table 2). Similar banding patterns were reported for Brassica species (Rabbani et al., 2001; Nasar et al., 2006; Turi et al., 2010).
SDS-PAGE has of paramount importance in separating and characterizing the proteins and estimating the extent of genetic diversity in the present set of garden cress germplasm. The banding pattern in the total seed protein showed close relationships among these studied genotypes. Similarity coefficient among these genotypes ranged from 25 to 100%. This is in agreement with earlier study on *Brassica carinata* where a similarity coefficient of 50 to 100% was reported (Zada et al., 2013).

Proteins have been used as markers for the assessment of genetic diversity in many crops (Iqbal et al., 2005; Nisar et al., 2016; Singh et al., 2017). The seed protein fragments exhibited appreciable polymorphism among the Ethiopian genotypes of garden cress, being used for the study of variability (Table 2). Consequently, electrophoretic analysis of the seed proteins had direct relationship to the genetic background of the proteins, and hence it is a potential marker for the study of genetic diversity and varietal identification. Similar studies have been carried out using protein marker for the study of genetic diversity and/varietal identification in many crops, as mentioned above (Iqbal et al., 2005; Netra and Prasad, 2007; Nisar et al., 2016; Singh et al., 2017). In general, DNA markers are more robust to detect variability among different genotypes. The only diversity study on garden cress using DNA markers was the one using less efficient inter simple sequence repeat (ISSR) (Said and Kassahun, 2015).

**Cluster analysis of seed storage proteins**

The cluster analysis showed that the genotypes were divided into three main groups (SG1-SG3) consisting of five major clusters and several sub-clusters (Figure 2). According to the magnitude of this genetic distance, genotypes from Cluster IV and V (SG3) were most divergent from the other two groups (SG1 and SG2). Such divergent genotypes should be used for designing effective breeding programs for evolving genetically vigor and variable breeding lines. Similarly, Cluster I and II represent relatively diverse group as compared to Cluster III. Thus, crossing between genotypes from Cluster I, II, III and genotypes from Cluster IV and V gene pools could create more genetic variability than crosses within genotypes of each main group (Figure 2). They are used
to develop desirable recombinant breeding lines and cultivars for future breeding programs.

Cluster III was the largest among all five clusters and consisted of 62 most similar genotypes (55% of the total), revealing low genetic diversity at genomic level (Table 3). However, the results might indicate the limitation on the number of markers used in the current study. In this connection, Opond-Konadu et al. (2005) reported the absence of large genetic difference among cowpea genotypes which hindered the use of protein electrophoresis to investigate diversity. Hence, genotypes in cluster III need to be further investigated in combination with 2D electrophoresis to minimize the lower variability detection efficiency of SDS-PAGE (Javaid et al., 2004; Jan et al., 2016). In this case, the integration of the usual electrophoresis separation with isoelectric focusing point electrophoresis to maximize the resolving power of seed storage protein markers due to amphoteric nature of amino acids is useful.

The cluster analysis also revealed that genotypes from different zones were observed to be closely related and genotypes from the same zone had different genetic background. This suggests that different selection pressures have been applied to yield and other biochemical properties in different genotypes. The high diversity among the genotypes from same region also shows the high exchange of germplasm among garden cress farmers although the exact mechanism of seed exchange among farmers from different regions has not yet been reported. According to Sihag et al. (2004) and Faisal et al. (2009) the cluster pattern for soybean genotypes showed that genetic diversity and geographic distribution were independent of each other and there was no definite relationship existed between them.

Table 2. The distribution and presence of bands in SDS-PAGE for 112 garden cress genotypes

| Region | Code of protein band | Number of genotypes | Present | Absent |
|--------|----------------------|---------------------|---------|--------|
| A      | 1                    | 56                  | 56      |
|        | 2                    | 41                  | 71      |
|        | 3                    | 48                  | 64      |
|        | 4                    | 49                  | 63      |
|        | 5                    | 94                  | 18      |
| B      | 6                    | 93                  | 19      |
|        | 7                    | 97                  | 15      |
|        | 8                    | 88                  | 24      |
|        | 9                    | 107                 | 5       |
| C      | 10                   | 104                 | 8       |
|        | 11                   | 99                  | 13      |
|        | 12                   | 108                 | 4       |
|        | 13                   | 101                 | 11      |
|        | 14                   | 65                  | 47      |
|        | 15                   | 101                 | 11      |
|        | 16                   | 90                  | 22      |
|        | 17                   | 106                 | 6       |
|        | 18                   | 103                 | 9       |
|        | 19                   | 112                 | 0       |
|        | 20                   | 112                 | 0       |
| Total  |                      | 1774                |         |
| Mean per genotypes |                      | 15.84               |         |
In general, the seed storage protein profiling generates a wide array of polymorphism, hence could serve as a valuable tool in determining the extent of genetic diversity. Thus, SDS-PAGE marker data provided more sub-groupings and revealed considerable amount of genetic diversity.

**Conclusions**

Based on similarity indices, the dendrogram divided the genotypes into three groups and five clusters, indicating the genetic relationships among genotypes. The grouping of genotypes into clusters did not associate with their geographic distribution. Seed storage protein profile could be economically useful marker to assess genetic diversity in garden cress germplasm. Predominately polymorphic proteins were noted in SDS-PAGE analysis used for selection of desirable genotypes in the garden cress improvement programs. However, the study revealed that nearly half of the genotypes were grouped into similar clusters requiring further analysis with a combination of 2D electrophoresis. The hybridization among the genotypes from distantly related groups is

---

**Table 3.** Grouping of tested genotypes using data derived from SDS-PAGE analysis.

| Cluster<sup>1</sup> | Accession ID                                      | Origin of genotype<sup>2</sup> |
|---------------------|--------------------------------------------------|---------------------------------|
| I (26)              | 241777, 225725, 219962, 212852, 240396, 229203, 237991, 229205, 90022, 212628, 90010, 90009, 229201, 240397, 231, 210, 229204, 208667, 215713, 90004, 18843, CG10, CG12, G16, CG18, CG19, CG20 | 1, 2, 3, 4, 5, 6               |
| II (10)             | 233984, 237512, 229798, CG2, CG17, 219960, 233982, 90016, 240578, 242609 | 1, 2, 3                           |
| III (62)            | 207542, 208693, 8604, 205141, 202116, 238273, 90006, 230831, 219959, 235892, 234355, 233983, 229202, 233370, 216885, 219958, 214243, 216814, 230829, 228200, 242916, 240579, 90002, 205163, 215714, 233985, 215807, 216866, 19001, 19000, 219961, 208030, 90021, 230523, 234828, 208666, 240808, 229199, 90018, 90014, 19002, 207910, 216815, 90005, 212853, 230830, 208669, 90007, 230524, 205162, 233981, CG1, CG4, CG5, CG6, CG7, CG8, CG11, CG13, CG15, CG21, CG22 | 1, 2, 3, 4, 5, 6               |
| IV (8)              | 90012, 233679, 225779, 229799, 90017, 18841, 90008, CG14 | 1, 2, 3, 6                       |
| V (6)               | 208769, 215808, 233986, 90020, CG3, CG9 | 1, 2, 5                         |

<sup>1</sup>Values in parenthesis indicate number of genotypes.

<sup>2</sup>Origin of genotype. 1, Amhara; 2, Oromia; 3, Southern Nation, Nationalities and of People Regional State (SNNP); 4, Somali; 5, Tigray; 6, unknown source.
suggested in order to enhance future breeding programs towards the development of desirable varieties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors would like to thank the Ethiopian Institute of Agricultural Research, particularly the National Agricultural Biotechnology Research Center at Holetta, Ethiopia, for providing necessary lab supplies and access to their lab facility. The Ethiopian Biodiversity Institute is also gratefully appreciated for the provision of garden cress accessions.

REFERENCES

Amare Getahun (1976). Some Common Medicinal and Poisonous Plants used in Ethiopian Folk Medicine. Faculty of Science, Addis Ababa University, Addis Ababa, Ethiopia.

Bemefo JEH, Leon J (1994). Neglected Horticultural Crops. Plant Production and Protection Series. FAO, Rome.

Bibi A, Rabbani MA, Abbasi FM, Niaz IA, Bibi K (2017). Assessment of storage proteins in indigenous rice accessions of northern Pakistan using biochemical markers. Pakistan Journal of Botany 49:155-161.

Bollag DM, Rozycki MD, Edelstein SJ (2002). Protein Methods, 2nd edition, Wiley-Liss, Inc. New York.

Buckseth T, Singh YV (2016). Seed storage protein profiling of pea (Pisum sativum L.) genotypes using SDS-PAGE. International Research Journal of Biological Sciences 5(1):37-41.

Choudhary R, Rai GK, Rai SK, Parveen A, Rai P, Salgotra RK (2015). Genetic diversity of Brassica napus using SDS-PAGE, SABRAO Journals of Breeding and Genetics 47(1):14-20.

Doke S, Guha M (2014). Garden cress (Lepidium sativum L.) seed - An important medicinal source: A Review. Journal of Natural Products of Plant Resources 4(1):69-80.

Faisal M, Malik A, Alsari S, Ashraf M, Khan MR, Javed A (2009). Evaluation of genetic diversity in soybean (Glycine max) lines using seed protein electrophoresis. Australian Journal of Crop Science 3(2):107-112.

Geetha VV, Balamurugan P (2011). SDS-PAGE electrophoresis in mustard cultivars. International Journal of Agriculture Research 6(5):437-443.

Gianazza E, Wait R, Sozzi RA, Regondi S, Saco D, Labra M, Agradi E (2007). Growth and protein profile changes in Lepidium sativum L. plantlets exposed to cadmium. Environmental and Experimental Botany 59:179-187.

Gokavi SS, Malleshi NG, Guo M (2004). Chemical composition of garden cress (Lepidium sativum L.) seeds and its fractions and use of bran as a functional ingredient. Plant Foods for Human Nutrition 59:105-111.

Hameed A, Qureshi M, Nawaz M, Iqbal N (2012). Comparative seed storage protein profiling of mung bean genotypes. Pakistan Journal of Botany 44(6):1993-1999.

Hameed A, Shah TM, Atta BM, Iqbal N, Haq MA, Ali H (2009). Comparative seed storage protein profiling of Kabuli chickpea genotypes. Pakistan Journal of Botany 41(2):703-710.

Iqbal J, Shinwari ZK, Rabbani MA, Khan SA (2014). Genetic variability assessment of maize (Zea mays L.) germplasm based on total seed storage proteins banding pattern using SDS-PAGE. East African Researches 2:2144-2160.

Iqbal SH, Ghafoor A, Ayub N (2005). Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. Pakistan Journal of Botany 37:87-96.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles 44: 222-275.

Jan SA, Shinwari ZK, Rabbani M A (2016). Determining genetic divergence among Brassica rapa ecotypes through electrophoretic mobility of total seed proteins. The Journal of Animal and Plant Sciences 26(6):1758-1764.

Javaid A, Ghafoor A, Anwar R (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pakistan Journal of Botany 30(1):25-29.

Kakaei M, Kahrizi D (2011). Evaluation of seed storage protein patterns of ten wheat varieties using SDS-PAGE. Biharesian Biologist 5(2):116-118.

Kancherla LS, Bhalla PM (2003). Phenotypic variations in micropropagated Australian ornamental climber Pandorea pandorana. Acta Horticulture 616:463-466.

Khan SA, Iqbal J, Khurshid H, Zia M, Shinwari ZK, Rabbani MA (2014). Intra and inter genetic diversity in rapeseed (Brassica napus L.) genotypes estimated through SDS-PAGE of total seed proteins. International Journal of Basic and Applied Sciences 3(2):110-117.

Laemmli UK (1970). Cleavage of structure proteins assembly of the head of bacteriophage T4. Nature 226:680-685.

Mandal RK, Mandal S (2000). Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. Current Sciences 79(5):576-589.

Manohar D, Viswanatha G, Nagesh S, Jain V, Shivaprashad H (2012). Ethno-pharmacology of Lepidium sativum L. (Brassicaceae): A Review. International Journal of Phytotherapy Research 2(1):1-7.

Nasar N, Khayami M, Heidari R, Jamei R (2006). Genetic diversity among selected varieties of Brassica napus (Cruferae) based on the biochemical composition of seeds. Jordan University of Science and Technology 32(1&2):57-68.

Netra N, Prasad S (2007). Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed proteins. Seed Science and Technology 35:176-186.

Nisar M, Ghafoor A, Wadood SF, Iqbal A, Nausheen A (2016). Intra and inter specific profiling of Pakistani Quercus species growing in the hilly areas of District Dir Khyber Pakhtunkhwa. Australian AGRONOMICAL CONFERENCES 48(1):263-270.

Opond-Konadu EYR, Akromah IK, Dapaah A, Okai E (2005). Genetic diversity within Ghanaian cowpea germplasm based on SDS-PAGE of seed proteins. African Crop Science Journal 13(2):117-123.

Parashar N, Jakhar LK, Krishna R, Jangid K (2015). Genetic diversity for storage seed protein profile in mustard [Brassica juncea (L.) CZern. & Coss.] genotypes. An International Quarterly Journal of Environmental Sciences 117:177-182.

Rabbani MA, Qureshi AA, Afzal M, Anwar R, Komatsu S (2001). Characterization of mustard (Brassica juncea L.) germplasm by SDS-PAGE of total seed proteins. Pakistan Journal of Botany 33:173-179.

Rahman MM, Hirata Y (2004). Genetic diversity in Brassica species using SDS-PAGE analysis. Journal of Biological Sciences 7(3):234-238.

Rava N (2016). A comprehensive review of Lepidium sativum L., a traditional medicinal plant. Review. World Journal of Pharmacy and Pharmacological Sciences 5(5):1593-1601.

Roy S, Kumar V (2014). A practical approach on SDS-PAGE for separation of protein. International Journal of Science and Research 3(8):955-960.

Sadia M, Malik SA, Rabbani MA, Pearce SR (2009). Electrophoretic characterization and the relationship between some Brassica species. Electronic Journal of Biology 5(1):1-4.

Said Mohammed and Kassahun Tesfaye. 2015. Molecular genetic diversity study of Lepidium sativum population from Ethiopia as revealed by inter simple sequence repeat (ISSR) markers. African Journal of Biotechnology 14:1461-1470.

Siddiqui MF, Naz N (2009). Protein landmarks for diversity assessment in wheat genotypes. African Journal of Biotechnology 8(8):1855-1859.

Sihag, Hooda JS, Vashishtha V, Joshi VA, Malik, BPS (2004). Genetic divergence in soybean [Glycine max (L.) Merril]. Annals of Biological Research 20(1):17-21.
Singh BK, Singh AK, Hotti AA, Kumar J, Singh SK (2017). Diversity analysis through SDS-PAGE of seed storage protein of pea genotypes. Research of Environmental and Life Sciences 10(5):449-452.

Singh CS, Paswan VK, Reeta BN (2015). Exploring potential of fortification by garden cress (Lepidium sativum L.) seed for development of functional foods – Review. Indian Journal of Natural Products and Resources 6(3):167-175.

Sharma DB, Krishna KR (2017). Genetic diversity in cowpea [Vigna unguiculata (L.) Walp.] accessions using protein profiling. International Journal of Pure and Applied Biosciences 5(2):491-496.

Temesgen B, Mebeasellassie A, Million E (2013a). Genetic variability and association among yield, yield related traits and oil content in Ethiopian garden cress (Lepidium Sativum L.) Genotypes. Journal of Plant Breeding and Crop Science 5:141-149.

Temesgen B, Mebeasellassie A, Million E (2013b). Genetic divergence analysis of garden cress (Lepidium sativum L.). Journals of Plant Breeding and Crop Sciences 5:770-774.

Turi NA, Rabbani MA, Khan NU, Akmal M, Pervaiz Z.H, Aslam MU (2010). Study of total seed storage protein in indigenous Brassica species based on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). African Journal of Biotechnology 9(45):7595-7602.

Wadhwa MS, Panwar A, Agrawal N, Patidar LN (2012). A Review on pharmacognostical study of Lepidium sativum L. Advanced Research in Pharmaceuticals and Biologicals 2(4):316-323.

Zada M, Shinwari ZK, Zakir N, Rabbani MA (2013). Study of total seed storage proteins in Ethiopian mustard (Brassica carinata A. Braun) germplasm. Pakistan Journal of Botany 45(2):443-448.