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Diversity Assessment of Jeera Accessions (*Nigella Sativa* L.), from Afghanistan and India, using Morphological and Yield Traits during Late *Rabi* 2014 and Early *Rabi* 2015

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ABSTRACT

Black cumin (N. sativa L.) is one such crop of great medicinal value, but less studied for the extent of variability in the available genetic resources and, for the influence of location and season on crop growth performance. The present study was undertaken to assess 37 accessions collected from Afghanistan and India. The accessions were sown during late Rabi 2014 and early Rabi 2015 and evaluated for 11 traits including yield and seed oil content. Black cumin accessions showed significant differences for all the characters including total yield and oil percentage during 2014. During 2015 the accessions did differ significantly for most characters except plant height, number of locules per capsule and seeds per capsule. Morphometric based PCA analysis revealed that there were broad-scale geographic genetic differentiation of the populations of N sativa and gene pools seem to be mixing well at a small geographic scale either because of human mediated commercial exploitation or because of natural outcrossing.

Key words: *Nigella sativa, Afghanistan, India, Diversity, PCA*

INTRODUCTION

Black cumin (*N. sativa* L. Family *Ranunculaceae*) is emerging as one such a miracle medicinal herb with rich historical and religious basis. The genus *Nigella* contains about 14 species of annual herbs of which *Nigella sativa*, is being used for healthcare from pre-historic times. It is commonly known as black cumin, fennel flower, Roman coriander and Kalonji[18]. *N. sativa* is native to the geographic space associated with Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries of Middle Eastern Mediterranean region, South Europe, and in India, Pakistan, Syria, Turkey, Saudi Arabia[1].

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The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. It is an important drug in the Indian traditional system of medicine like Unani and Ayurveda[2,4]. Avicenna[3] refers to black seeds in the ‘’The Canon of Medicine’’, as seeds that stimulate the body’s energy and helps recovery from fatigue and dispiritedness. Black seeds and their oil have a long history and folklore of usage as food and medicine in Indian and Arabian civilization[7,8]. Black cumin oil is the spicy yellowish oil with an aromatic flavor; fresh flavored odor is extracted from black cumin seed[9,13]. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. Black seeds are also used as flavoring additive in the breads and pickles as it has very low of toxicity[6].

MATERIALS AND METHOD

Thirty seven accessions collected from different places from Afghanistan (28 accessions) and India (Nine accessions) were included in this study. The accessions from Afghanistan were collected from the field visits to different places of cultivation (Figure 1), while the accessions from India were procured from the markets at Bengaluru. Therefore the exact location of origin of the latter cannot be known. Collected germplasms were planted in randomised complete block design (RCBD) with three replications in late *Rabi* 2014 and two replicatons in early *Rabi* 2015 under file condition repectivelly at Department of Genetics and Plant Breeding (13°05'06.6"N 77°34'30.7"E), University of Agricultural Sciences, GKVK, Bengaluru, and department of Plantation, Spices, Medicinal, and Aromatic Crops (13°05'45.5"N 77°33'54.0"E), College of Horticulture, GKVK, Bengaluru . The experimental site was ploughed three times and brought to a fine tilth and the land was divided into plots of required size. All other cultural practices were followed to maintain proper growth and development of crop up to harvesting time. The observations days to first flowering; days to 50% flowering; plant height (cm); number of branches per plant; number of capsules per plant; ten capsules weight; number of locules per capsule; number of seeds per capsule; 1000 seeds weight (mg); seed yield per plant (g) and oil content were recorded on the selected plants in each replication and average was calculated.

RESULTS

The mean performance of all accessions was though similar during the both seasons for many traits, the plants grew taller (62.83 cm) during early 2015 compared to late *Rabi 2014* (38.57 cm). Average yield and oil percentage were very different between two years; the average seed yield was more (2.31 g per plant) during late *Rabi 2014* compared to 2015 (1.93 g per plant) and the average oil percentage was 23.25% during late *Rabi 2014* and it was 21.33% during *Rabi* 2015. Day to first flowering ranged from 53.20 to 56.06 days, days to 50% flowering ranged from 66.66 to 78.33 days, plant height ranged from 28.81 to 46.4 cm, number of branches per plant ranged from 16.85 to 28.26, number of capsules per plant ranged from 18.76 to 31.93, ten capsules weight ranged from 1.31 to 2.19 g, number of locules per capsule ranged from 5.43 to 6.38, number of seeds per capsule ranged from 71.27 to 90.30, 1000 seeds weight ranged from 1534.58 mg to 1680.41mg, seed yield per plant ranged from 1.44g to 3.35g, oil percentage ranged from 13.76% to 30.12% during late *Rabi* 2014.

During early *Rabi* 2015, except four traits viz; days to first flowering, plant height, number of locueles per capsule and seeds per capsule all other traits showed significant differences. The seed yield per yield manifested highest (F) ratio (21.05), followed by 1000 seeds weight (16.12) and 10 capsules weight (10.16). The days to first flowering ranged from 59.30 to 63.20 days, days to 50% flowering ranged from 69.50 to 77.00 days, plants height ranged from 21.08 to 27.81 cm, number of branches per plant ranged from 19.85 to 28.55, number of capsules per plant ranged from 17.37 to 26.55, ten capsules weight ranged from 1.38 to 2.24 g, number of locules per capsule ranged from 4.38 to 5.28, number of seeds per capsule ranged from 69.91 to 83.33, 1000 seeds weight ranged from 1517.62 to 1651.00 mg, seed yield per plant ranged from 1.28 to 2.23g , oil percentage ranged from 13.63 to 26.62 (Table 1-a and Table 1-b). Highly significant difference was observed among accessions for all the characters during late *Rabi 2014*.

Moreover, even in the weights assigned to different traits also there was a similarity between the two years, especially when the first two PC Axes are considered (Figure 2 and Figure 3). For example the most important traits that contributed through the first Axis during 2014 were 1000 seed weight (-0.52), plant height (0.50), and number of locules per capsule (-0.48) while through the second axis, number of branches per plant (0.48) and seed yield per plant (0.48) contributed the most. During the 2015 season, the traits that contributed the most were number of capsules per plant (0.51), number of branches per plant (0.42) and 10 capsule weight (0.37) through the first PC Axis while seed yield per plant (-0.58), oil per cent (0.44) and days to 50 % flowering (-0.43) contributed through the second PC Axis. The Principal Component Analysis (PCA) could extract only 84.18 and 84.12 % of the variability among the accessions for all the traits (Figure 4 and Figure 5). We used only the first three iterations as they together extracted more than 50 % variability. These first three Principal Component Axes (PCA) could extract 55.79 % during 2014 and 53.78 during 2015. Again the extent of each of the Axes extracted was also similar during both the years. During the years 2014 and 2015, the first PCA extracted 21.41 and 21.77 %, the second PCA extracted 18.66 and 17.22% and the third PCA 15.72 and 14.79 % respectively. The separation of the accessions in the space between the first two axes was also very similar in both the years. There are three distinct patterns that can be discerned from the spatial separation of the accessions between the first two PC Axes: The 37 accessions separated widely during both the years suggesting that the collections from India and Afghanistan are clearly distinct accessions with less genetic overlap among them. The Indian and Afghanistan accessions showed a clear separation on the PCA space. All the Indian accession can be seen to be congregated in the space defined by the mid values of PCA- I and high values of PCA II, as shown enclosed in the marked shape in Figure 4. Similarly, the 2015 analysis also showed a clear separation in to the space defined by the higher values of PCA- I and low values of PCA-II. Rarely did the Afghan collections were mixed with the Indian accessions except for INB-M4. In fact INB-M4 that grouped with Afghan accessions did so during both the years (Figure 5).The pattern of separation of accessions was consistent in both the years: For example Accessions AFN-KA and AFB-PU were the most divergent accessions in the PCA analysis of both the seasons. The two occupied distinctly opposite spectra of the space. Further some of the accessions that grouped together during the 2014 such as AFK-IM and AFF-QA occupied the neighborhood during both the seasons.

Table 1-a: Analysis of variance for different traits among the selected genotypes in late *Rabi* 2014

	Days to first flowering	Days to 50% flowering	Plant height (cm)	Number of branches per plant	Number of capsules per plant	10 capsules weight	Number of locules per capsule	Number of Seeds per Capsule	1000 seeds weight (mg)	Seed yield Per plant (g)	Oil content %
Mean	**54.58**	**73.12**	**38.57**	**22.83**	**25.54**	**1.87**	**5.88**	**80.72**	**1587.13**	**2.31**	**23.25**
C.V.	1.04	3.19	4.33	7.51	5.85	8.72	5.55	5.01	1.26	10.42	3.95
F ratio	6.00**	3.82**	15.36**	7.23**	9.77**	3.81**	1.64*	5.26**	10.00**	12.16**	68.53**
S.E.	0.33	1.35	0.96	0.99	0.86	0.09	0.19	2.34	11.51	0.14	0.53
C.D. 5%	0.92	3.80	2.72	2.79	2.43	0.26	0.53	6.58	32.44	0.39	1.49
C.D. 1%	1.23	5.04	3.61	3.70	3.23	0.35	0.71	8.74	43.06	0.52	1.98
Range	**53.20-56.06**	**66.66-78.33**	**28.81-46.44**	**16.85-28.26**	**18.76-31.93**	**1.31-2.19**	**5.43-6.38**	**71.27-90.30**	**1534.58-1680.41**	**1.44-3.35**	**13.76-30.12**

Table 1-b: Analysis of variance for different traits among the selected genotypes in early *Rabi* 2015

	Days to first flowering	Days to 50% flowering	Plant height (cm)	Number of branches per plant	Number of capsules per plant	10 capsules weight	Number of locules per capsule	Number of Seeds per Capsule	1000 seeds weight (mg)	Seed yield Per plant (g)	Oil content %
Mean	**61.30**	**72.89**	**62.83**	**24.37**	**21.84**	**1.85**	**5.38**	**78.64**	**1583.88**	**1.93**	**21.33**
C.V.	1.79	1.85	462.22	4.53	5.02	4.66	4.71	860.23	0.64	3.75	3.28
F ratio	1.49NS	3.86**	1.00NS	7.72**	8.64**	10.16**	1.33NS	1.00NS	16.12**	21.05**	44.74**
S.E.	0.78	0.95	205.35	0.78	0.78	0.06	0.7 206	2.07	7.12	0.05	0.49
C.D. 5%	-	2.73	-	2.24	2.23	0.17	-	-	20.42	0.15	1.42
C.D. 1%	-	3.66	-	3.00	2.98	0.23	-	-	27.39	0.20	1.90
Range	**59.30-63.20**	**69.50-77.00**	**21.08-27.81**	**19.85-28.55**	**17.37-26.55**	**1.38-2.24**	**4.38-5.28**	**69.91-83.33**	**1517.62-1651.00**	**1.28-2.23**	**13.63-26.62**

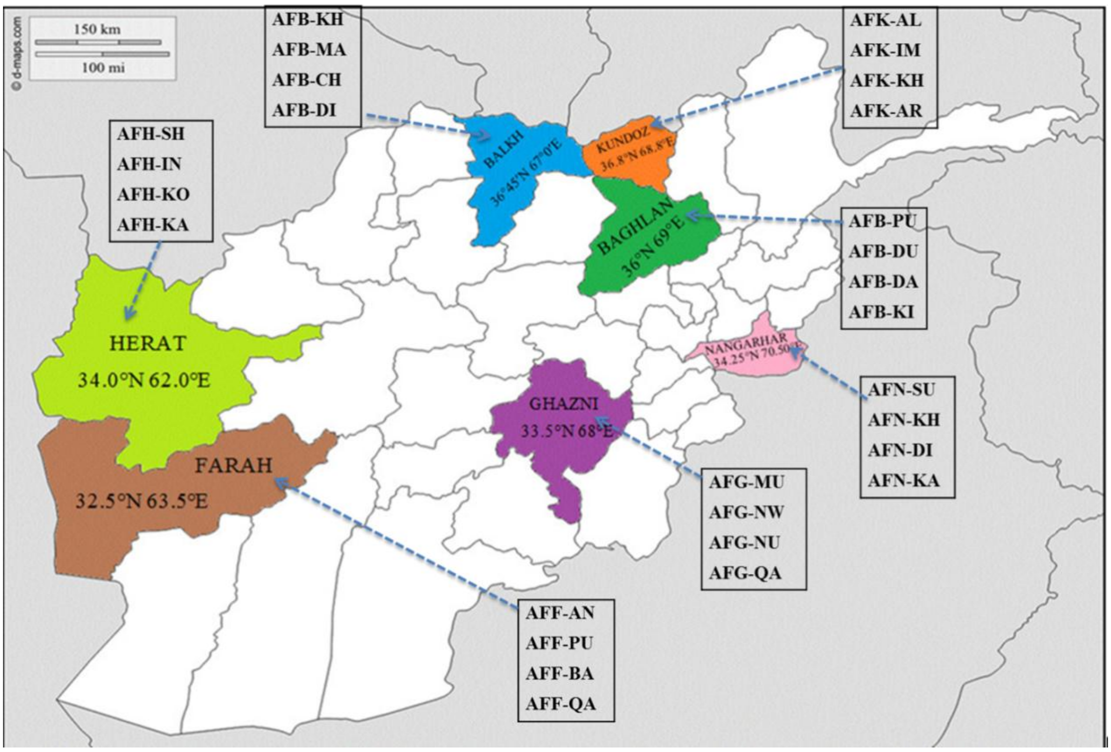

Fig. 1: Collection areas of *Nigella sativa* L. accessions in Afghanistan

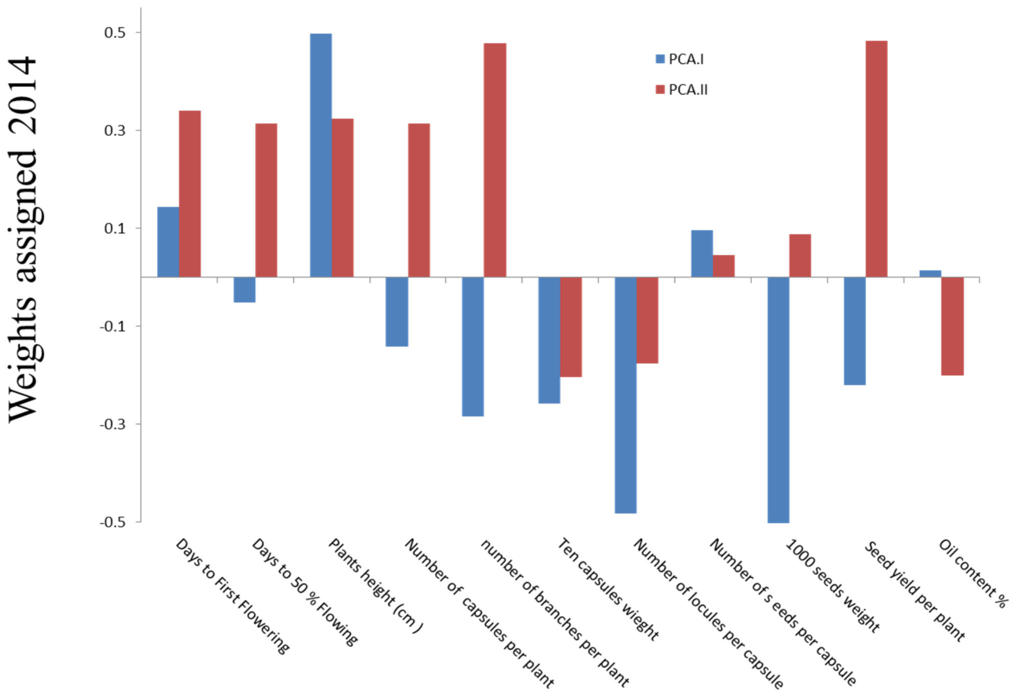

Fig. 2: Graphical representation of PCA weightages assigned to variables late *Rabi* 2014

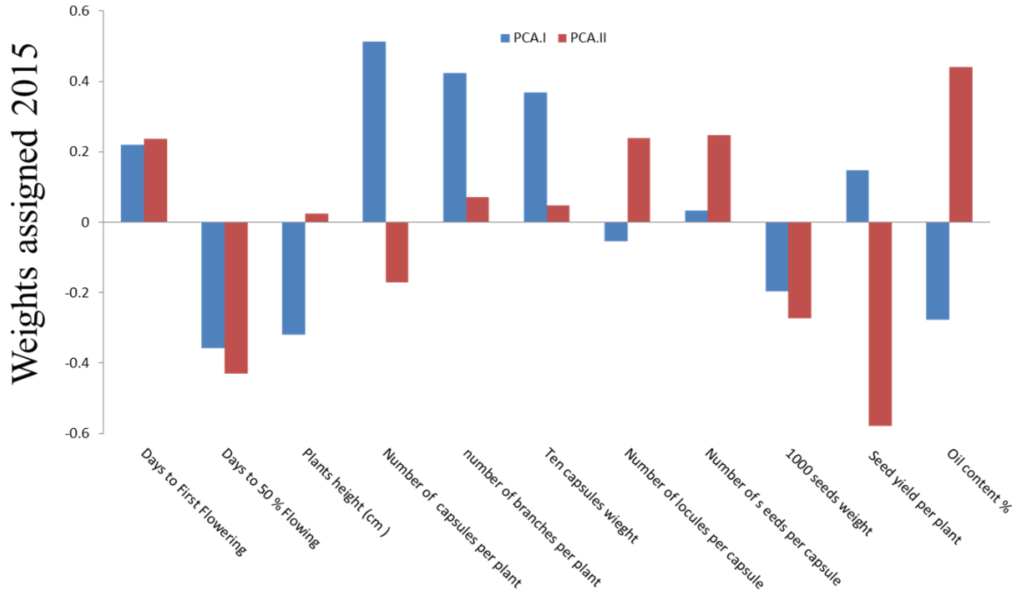

Fig. 3: Graphical representation of PCA weightages assigned to variables early *Rabi* 2015

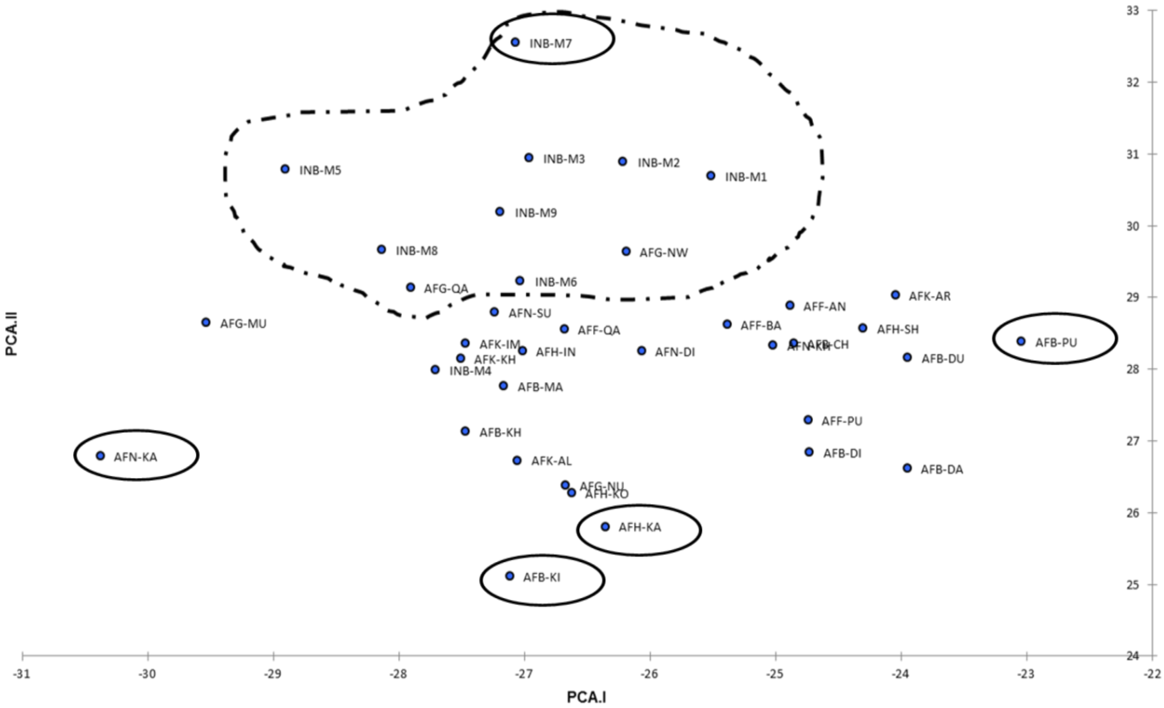

Fig. 4: Distribution of accessions between the first two PC Axes (late *Rabi* 2014). The circled accessions are distinct types. The dotted shape encloses accessions mostly from India

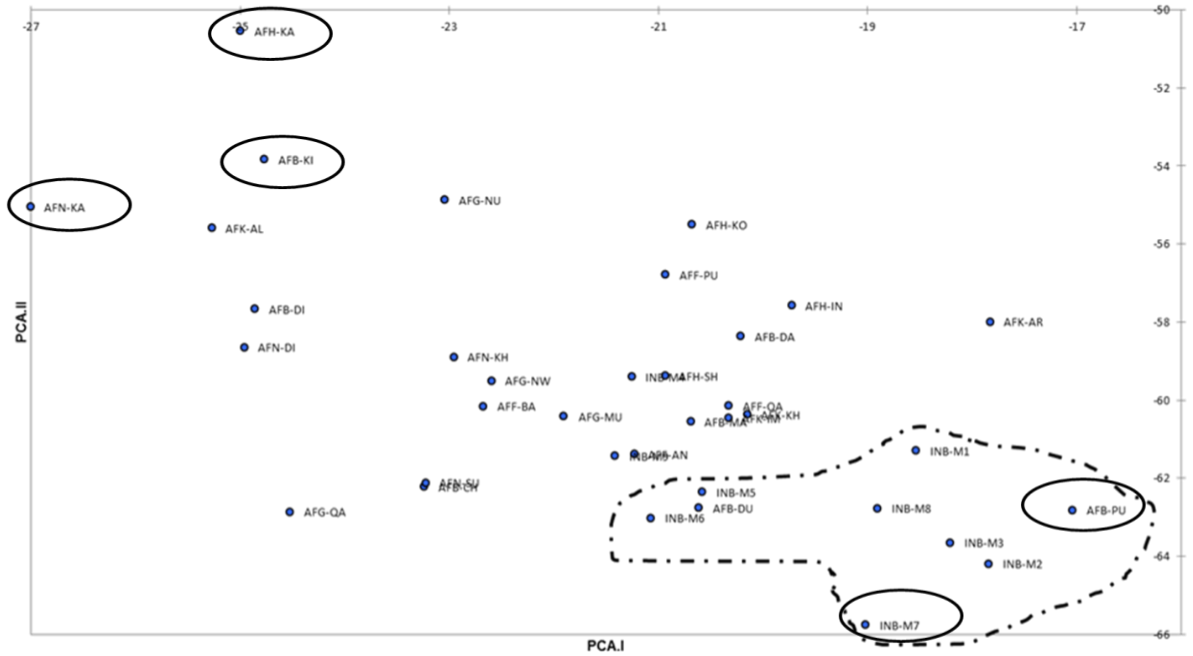

Fig. 5: Distribution of accessions between the first two PC Axes (early *Rabi* 2015). The circled accessions are distinct types. The dotted shape encloses accessions mostly from India

DISCUSSION

The 37 accessions showed significant differences for all the characters including seed yield per plant and oil content during late *Rabi* 2014. During early *Rabi* 2015 the accessions did differ significantly for most characters except plant height, number of locules per capsule and seeds per capsule. Such difference in the performance of crops in different seasons has been reported in other medicinal plants such as *Adhatoda vasica*[12], *Mentha pulegium*[16], *Sargassum wightii*[14], *Mentha spicata*[11], *Toona sinensis*[17], *Adiantum capillus-veneris*[5], *Ocimum basilicum*[10] and *Ulva reticulata*[15]. The highest yielding accession (INB-M7; 3.35 g) was 2.33 times more than least yielding accession (AFH-KA; 1.44 g) during *Rabi* 2014. However this range was only 1.74 times the least yielding accession during early *Rabi* 2015. Similarly the range of oil percentage was also higher (2.18 times than lowest oil percentage) during late *Rabi 2014* compared to early *Rabi* 2015 (1.95 times). It seems by focussing on yield and oil percentage it would be possible to attain substantial improvement in yield of the crop. Accordingly it appears profitable to suggest the cultivation of the lines INB-M7 and AFF-PU that have yielded highest seed per plant and oil percentage.

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