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# GENETIC VARIATION IN YIELD PERFORMANCE FOR THREE YEARS IN *NIGELLA SATIVA* L. GERMPLASM AND ITS ASSOCIATION WITH MORPHO-PHYSIOLOGICAL TRAITS AND BIOCHEMICAL COMPOSITION

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**Abstract**

In the present study, thirty two genotypes of *Nigella sativa* L., germplasm were evaluated for various agriculturally important traits for 3 consecutive years along with biochemical composition. Low variance was recorded in four genotypes viz., Pk-020545, Pk-020561, Pk-020576 and Pk-020646 that indicated stability. Performance of the most of the genotypes was affected by environmental fluctuations that might be attributed to cultural practices or genetic inference. High genetic variability and heritability provide an estimate and opportunity for breeders to select the promising genotypes for specific traits. The association of oil content and fatty acid methyl esters profile (oil extracted with *n*-hexane) revealed that linoleic acid was significant with yield during first year. Oil content, oleic acid, linoleic acid, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (with *n*-hexane) were significant during second year. Zn was significantly associated with yield obtained in the third year traits with high significance could be exploited for further hybridization and breeding program is suggested. By employing biotechnological tools in preparation of effective and safe products can be obtained, additionally they could play important role in medicinal plants cultivation, new sources of production of bioactive secondary metabolites and other aspects of medicinal plant biochemistry.

## Introduction

*Nigella sativa* L. or black seeds, a member of *Rananculaceae* (diploid, 2n=12) is a minor cultivated crop from Morocco to Northern India; in sub-Saharan Africa particularly Niger, and eastern Africa especially Ethiopia (Iqbal *et al*., 2010). It has been used for healthcare since civilization in Egypt, Iran, India, Pakistan, Saudi Arabia, Syria, Turkey and many other countries. Many medical properties have been attributed to the *Nigella sativa* L. seeds and its oil, including carminatives, diuretics, antineoplastic (antitumour), antifungal, anti-helminthic, while their oil has protective action against histamine induced bronchospasm, cough and bronchal asthma (Worthen *et al*., 1998; Khan *et al*., 2003), antidiabetics (Fararh *et al*., 2002; El-Dakhakhny *et al*., 2002), spasmolytic and bronchodilator (Boskabady *et al*., 2004), anti-inflammatory (Hajhashemi *et al*., 2004), antibacterial (Mashhadian & Rakhshandeh, 2005), galactogogue, antioxidant (Brutis & Bucar, 2000; Kanter *et al*., 2003) and insect repellent effects (Fisher, 2002).

More than 100 chemical constituents have been identified in *Nigella sativa* L. up till now (Iqbal, 2008). The black seed contains a non-starch polysaccharide component which is a useful source of dietary fiber. It is rich in fatty acids, particularly the monounsaturated, unsaturated and essential fatty acids (Oleic acid, Linoleic and Linolenic acid) (Ramadan & Morsel, 2003). Amino acid analysis of seeds protein hydrolysate showed the presence of 15 amino acids of which amazingly includes all 9 essential amino acids (Al-Awadi, 1998). Additionally, black seed is a valuable source of protein, carbohydrates, essential fatty acids, vitamins A, B1, B2, C and niacin as well as minerals such as calcium, potassium, iron, magnesium, selenium, manganese and zinc. These are also known to contain essential macro and micronutrients which play vital role as structural and functional components of metalloproteins and enzymes (co-factors) in the living cells (Anonymous, 2006; Ansari *et al*., 2004). *Nigella sativa* L. is being considered important for both oil and bioactive compounds because their constituents have unique chemical properties and may augment the supply of edible oils (Ramadan & Morsel, 2003). So far, a large number of plants have been analysed and some of these have been cultivated as new oil crops (Hirsinger, 1989).

*Nigella* seed has higher total phospholipid content than cotton and soybean seed oil (Ramadan and Morsel, 2003; Atta, 2003; Gunstone *et al*., 1986). Phosphatidyl choline, sphingomyelin, phosphatidyl serine, phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidyl glycerol and cardiolipin were identified in *Nigella sativa* L. seeds (Ramadan & Morsel, 2003) and also quantified by Iqbal (2008). Phosphatidyl choline and lyso-phosphatidyl choline are known to be important in membrane properties such as synthesis of lipid bilayer and liposome formation. They are also useful as emulsifiers in food and pharmaceutical applications. In *Nigella* oil, phospholipids, particularly those with free amino groups, may interact as a natural antioxidant and consequently increase oil stability and shelf life (Ramadan & Morsel, 2003; Boyd, 2001; Rathjen & Steinhert, 1997).

Variances of quantitative genetic traits provide an estimate of genetic diversity (Ghafoor *et al*., 2001). Therefore, evaluation of available genetic stocks to assess the genetic variation for economically important characteristics is a pre-requisite for combining desirable genes in a single genotype (Palevitch, 1987). The objectives of this study were to predict extent of genetic-biodiversity by evaluation and characterization of morpho-physiological traits, nutritional characteristics, mineral nutrients, fatty acid and phospholipid profile and their association with yield over three years.

## Materials and Methods

*Nigella sativa* L. germplasm was planted under field conditions of Plant Genetic Resources Program, National Agricultural Research Center, Islamabad, Pakistan ($33^0$ 44' N $73^0$ 08' E 540 masl). Among 32 genotypes, 30 were collected locally and two genotypes were received from Western Regional Plant Introduction Station, United States Department of Agriculture, Maryland, Washington, USA. The experiment was planted in randomized complete block design (RCBD) with four replications during 2008-2009, 2009-2010 and 2010-2011. Three rows of each genotype were planted in 4 meter bed length, 20 cm plant to plant distance, whereas row distance was 30cm. Fifty cm distance was kept to separate two genotypes. Form yard manure was applied and all other cultural practices were followed throughout the crop season (Anon., 2008).

Morpho-physiological traits days to first flower, days to 50% flowers, days to maturity, flowering duration were recorded on plot basis while plant height, biological yield, number of branches, capsule weight, number of capsules, capsule length, capsule width, number of locules, root weight, root length, 1000 seed weight and grain yield were recorded on 10 randomly sampled plants within each genotype. For 1000 seed weight, seeds from the single plant were harvested and weighed. Ten capsules from each plant were sampled at random for recording of capsule length, capsule width and number of locules. Harvest index was expressed as a ratio between grain yield and biological yield. Physiological traits (trait name, notion/codes and description of the traits) recorded are presented in the Table 1. For oil extraction by Soxtec system 3 solvents (*n*-hexane, CM 2:1 and CM 1:3) were used and the separation and identification of fatty acid methyl esters were achieved by injecting standard Rape-Mustard (RM-2) into GLC to get the particular spectra. Phospholipids were quantified by normal phase HPLC into 10 components (Lysophosphatidyl choline, phosphatidyl choline, sphingomyelin, phosphatidyl serine, phosphatidyl inositol, lyso-phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl glycerol and cardiolipin). The nutritional characteristics and minerals were estimated by following standard methods by Anon., (2005). The data were analyzed for analysis of variance (ANOVA) by MSTATC, mean and variance by using computer software MS Excel for Windows XP.

**Table 1. Plant morpho-physiological traits studied in 32 genotypes of *Nigella sativa* L., germplasm.**

| Traits | Notions/codes | Description of the traits |
|---|---|---|
| Days to first flower | D1F | Days from the time of planting to the appearance of first flower |
| Days to 50% flowers | D50%F | Days from the time of planting to the appearance of 50% flowers |
| Days to maturity | DM | At the stage of capsule maturity |
| Flowering duration | FD | Days between days to first flower and 50% flowers |
| Plant height | PH | The length of the upper part above the ground (length of the plant stem) |
| Biomass | BM | The weight of the whole plant except roots |
| Number of branches | NB | The total number of branches |
| Capsule weight | C wt | The weight of the capsules |
| Number of capsule | NC | The total number of capsules present on the particular plant/plants |
| Capsule length | CL | Length of capsules |
| Capsule width | CW | The width of the capsules |
| Number of locules | NL | The total number of locules (where seeds are formed/placed) |
| Root weight | RW | The weight of the root |
| Root length | RL | The length of the root |
| Seed weight | 1000 SW | The weight of the seeds (the 1000 seeds weight) |
| Grain yield | GY | The production/weight of the grains/seeds for particular plant |
| Harvest index | HI | The difference between grain yield over biomass, multiplied by 100 |

## Results and Discussion

High diversity was observed for grain yield during all the 3 years i.e., 2008-09, 2009-10 and 2010-11 as presented in Table 2. Significant differences were revealed for yield within years although the level of variation was low in the third year. Low variance was recorded in four genotypes Pk-020561, Pk-020576, Pk-020545 and Pk-020646 that indicated higher level of stability. It revealed the worth of genetic diversity for stability that can be exploited in this crop.

Association of oil contents and fatty acid methyl esters profile revealed that linoleic acid (oil extracted with *n*-hexane), was significant with yield during 2008-09 (Table 3). Oil content, oleic acid, linolenic acid, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (with *n*-hexane) while oil content, palmitic acid, linoleic acid, saturated fatty acids (SFA), unsaturated fatty acids (UNSF), and polyunsaturated fatty acids (PUFA) were found significant association with 2009-10 (oil extracted with CM 1:3). Palmitic acid (CM 1:3), linolenic acid (*n*-hexane), saturated fatty acids (SFA) (CM 1:3), unsaturated fatty acids (USFA) (CM 1:3) were found significant with yield obtained in 2009-10. The Table 4 presents the coefficient of correlation for phospholipids (PLs), nutritional characteristics and mineral nutrients with yield obtained during three years. The phospholipids; PG, CL, PE, LPE and Pb were found significant association with yield during 2008-09. On the other hand, protein, carbohydrates, moisture, Na and K were observed significant association with yield obtained in 2009-10. Zn was significantly associated with yield recorded during 2010-2011.

**Table 2. Variation in yield performance for three years in 32 genotypes of *Nigella sativa* L., germplasm.**

| Traits | Yield during | | | |
|---|---|---|---|---|
| | 2008-09 ± SD | 2009-10 ± SD | 2010-11 ± SD | Average yield ± SD |
| PK-020561 | 8.88 ± 0.32 | 11.05 ± 0.24 | 2.13 ± 0.40 | 7.35 ± 2.49 |
| PK-020576 | 6.73 ± 0.54 | 13.01 ± 6.34 | 1.73 ± 0.22 | 7.16 ± 2.91 |
| PK-020545 | 3.33 ± 0.37 | 8.65 ± 1.27 | 2.23 ± 0.70 | 4.73 ± 2.94 |
| PK-020646 | 6.40 ± 1.15 | 8.55 ± 1.32 | 2.03 ± 0.23 | 5.66 ± 2.99 |
| PK-020620 | 6.64 ± 0.50 | 10.55 ± 2.60 | 2.17 ± 0.98 | 6.45 ± 3.34 |
| PK-020567 | 4.58 ± 3.03 | 15.67 ± 4.48 | 2.82 ± 0.79 | 7.69 ± 3.37 |
| PK-020720 | 2.81 ± 0.82 | 8.52 ± 1.04 | 2.50 ± 0.69 | 4.61 ± 3.44 |
| PK-020663 | 4.20 ± 3.32 | 11.65 ± 1.70 | 2.43 ± 0.99 | 6.10 ± 3.47 |
| PK-020585 | 4.42 ± 1.79 | 15.22 ± 5.45 | 2.45 ± 0.88 | 7.36 ± 3.58 |
| PK-020631 | 3.53 ± 1.51 | 7.19 ± 3.41 | 2.07 ± 0.50 | 4.26 ± 3.58 |
| PK-020742 | 3.33 ± 0.67 | 6.32 ± 2.13 | 1.96 ± 0.99 | 3.87 ± 3.59 |
| PK-020592 | 3.69 ± 0.59 | 8.84 ± 2.85 | 2.79 ± 0.21 | 5.11 ± 3.67 |
| PK-020867 | 3.82 ± 0.77 | 4.70 ± 4.99 | 2.37 ± 0.39 | 3.63 ± 3.70 |
| PK-020780 | 4.95 ± 0.49 | 6.93 ± 4.77 | 3.07 ± 1.01 | 4.98 ± 3.74 |
| PK-020609 | 4.42 ± 1.92 | 9.17 ± 1.37 | 2.60 ± 0.29 | 5.39 ± 3.74 |
| PK-020654 | 4.41 ± 0.77 | 9.88 ± 2.39 | 2.57 ± 0.64 | 5.62 ± 3.80 |
| PK-020872 | 7.57 ± 0.24 | 9.13 ± 2.04 | 2.50 ± 0.82 | 6.40 ± 3.82 |
| PK-020766 | 5.57 ± 0.60 | 7.55 ± 1.85 | 2.25 ± 0.12 | 5.13 ± 3.83 |
| PK-020781 | 3.90 ± 0.90 | 9.94 ± 2.35 | 2.75 ± 0.36 | 5.53 ± 3.87 |
| PK-020749 | 5.20 ± 0.38 | 7.79 ± 6.85 | 2.68 ± 0.36 | 5.22 ± 3.88 |
| PK-020662 | 6.64 ± 0.65 | 10.02 ± 1.10 | 2.59 ± 0.16 | 6.42 ± 3.97 |
| PK-020729 | 5.21 ± 0.87 | 8.70 ± 2.31 | 2.22 ± 0.77 | 5.38 ± 3.97 |
| PK-020871 | 3.39 ± 1.09 | 7.39 ± 3.07 | 2.04 ± 0.23 | 4.27 ± 4.01 |
| PK-020868 | 5.27 ± 0.48 | 4.88 ± 2.69 | 2.14 ± 0.71 | 4.10 ± 4.04 |
| PK-020699 | 4.13 ± 0.62 | 9.62 ± 2.04 | 2.38 ± 0.73 | 5.38 ± 4.05 |
| PK-020783 | 4.40 ± 0.91 | 6.24 ± 1.76 | 2.49 ± 0.16 | 4.37 ± 4.06 |
| PK-020875 | 4.17 ± 0.10 | 12.27 ± 3.84 | 2.57 ± 0.40 | 6.34 ± 4.14 |
| PK-020874 | 6.11 ± 1.13 | 9.99 ± 2.60 | 2.52 ± 0.19 | 6.20 ± 4.45 |
| PK-020873 | 4.95 ± 0.86 | 9.18 ± 4.90 | 2.21 ± 0.24 | 5.45 ± 4.54 |
| PK-020876 | 4.15 ± 0.62 | 8.23 ± 2.32 | 2.66 ± 0.16 | 5.01 ± 4.60 |
| PK-020878 | 5.84 ± 1.12 | 8.04 ± 1.61 | 2.18 ± 0.51 | 5.35 ± 5.01 |
| PK-020877 | 3.69 ± 0.73 | 7.46 ± 0.71 | 2.56 ± 0.28 | 4.57 ± 5.03 |
| MS (V/T) | 19.45 | 79.32 | 0.77 | |
| MS (Rep) | 3.941 | 19.07 | 0.08 | |
| MS (Error) | 1.28 | 6.93 | 0.36 | |
| Standard Error | 0.57 | 1.32 | 0.30 | |
| CDI | 1.58 | 3.69 | 0.84 | |
| CD2 | 2.09 | 4.87 | 1.11 | |
| H | 0.78 | 0.72 | 0.22 | |

SD, Standard deviation; MS, Mean Square; V/T, Varieties/Treatments; Rep, Replicates; CD1, Critical difference; CD2, Critical difference; H, Heritability in broader sense

Morpho-physiological traits have long been used by plant breeders to study genetic variation patterns and correlations in populations. These methods involve a lengthy survey of plant growth that is costly, labour intensive, and vulnerable to environmental conditions but due to their importance these are still considered as the first step in any crop improvement program (Iqbal *et al*., 2010). Management practices and human interpretation also have influence on phenotypic expressions (Coetzee, 2004). Since *Nigella sativa* L. is a new crop to Pakistan that may be planted on commercial scale for seed and oil production in future (Iqbal, 2008).

For morpho-physiological traits, the genotypes differed significantly for all the traits recorded indicating a considerable range of genetic variability during all the three years (Iqbal *et al*., 2013). Maximum grain yield was produced by the genotype Pk-020878 while the lowest by Pk-020545 during 2008-09. During 2004, the highest grain yield was recorded in Pk-020654 and lowest was in Pk-020720 whereas during third year, the highest grain yield was obtained in Pk-020780 and the lowest was in Pk-020781. Data showed that all these genotypes performed differently and the yield over the years was affected by environmental factors or this might be attributed to cultural practices performed during field evaluation, as this was already reported in *Nigella sativa* L., by Cheikh-Rouhou, (2007) and Atta, (2003). High genetic variability and heritability of the characters provide an estimate for genetic diversity and opportunity to the breeders to select promising genotypes for specific traits. Although, phenotypic variation is based on multiple factors, analysis of germplasm provided a method of evaluation and identification of land races that could be further characterized by DNA molecular markers is also suggested (Iqbal *et al*., 2011; Bibi *et al*., 2012).

**Table 3. The coefficient of correlation for yield with oil contents and fatty acid profile extracted by three solvents (*n*-Hexane, CM (2:1) and CM (1:3) in 32 genotypes of *Nigella sativa* L., germplasm.**

| Oil and fatty acid methyl esters profile | Yield during | | | |
|---|---|---|---|---|
| | Solvents | 2008-09 | 2009-10 | 2010-2011 |
| | *n*-Hexane | 0.20 | 0.34* | 0.05 |
| Oil contents | CM (2:1) | -0.05 | 0.13 | 0.14 |
| | CM (1:3) | 0.02 | -0.39* | -0.11 |
| | *n*-Hexane | 0.23 | -0.29 | -0.15 |
| Palmitic acid | CM (2:1) | -0.11 | -0.26 | 0.23 |
| | CM (1:3) | 0.03 | -0.38* | 0.37* |
| | *n*-Hexane | 0.14 | -0.02 | -0.01 |
| Stearic acid | CM (2:1) | -0.19 | -0.10 | -0.02 |
| | CM (1:3) | -0.10 | -0.05 | 0.24 |
| | *n*-Hexane | 0.16 | -0.36* | 0.08 |
| Oleic acid | CM (2:1) | 0.21 | 0.07 | 0.08 |
| | CM (1:3) | 0.03 | -0.17 | -0.19 |
| | *n*-Hexane | -0.34* | 0.44* | 0.15 |
| Linoleic acid | CM (2:1) | -0.03 | 0.06 | -0.25 |
| | CM (1:3) | -0.02 | 0.34* | -0.16 |
| | *n*-Hexane | 0.32* | -0.21 | -0.37* |
| Linolenic acid | CM (2:1) | -0.09 | 0.12 | 0.22 |
| | CM (1:3) | -0.25 | -0.02 | 0.09 |
| | *n*-Hexane | 0.24 | -0.24 | -0.13 |
| SFA | CM (2:1) | -0.14 | -0.27 | 0.21 |
| | CM (1:3) | 0.00 | -0.35* | 0.39* |
| | *n*-Hexane | -0.22 | 0.26 | 0.13 |
| USFA | CM (2:1) | 0.15 | 0.22 | -0.24 |
| | CM (1:3) | -0.03 | 0.32* | -0.39* |
| | *n*-Hexane | 0.16 | -0.36* | 0.08 |
| MUFA | CM (2:1) | 0.17 | 0.20 | 0.15 |
| | CM (1:3) | 0.03 | -0.17 | -0.19 |
| | *n*-Hexane | -0.26 | 0.41* | 0.03 |
| PUFA | CM (2:1) | -0.05 | 0.09 | -0.20 |
| | CM (1:3) | -0.04 | 0.34* | -0.15 |

**Highly Significant, *Significant at 0.01% and 0.05% probability level ; SFA, Saturated fatty acids; USFA, Unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

During 2009-10, the crop matured late as compared to 2008-09 and 2010-11 that might be due to environmental influence. There was considerable differentiation in the performance of traits during three years which might be influenced by the environmental factors; seed handling, harvesting and preservation conditions. Atta (2003) advocated this in reference with *Nigella sativa* L. that such variation in nutrient concentrations among species and varieties may be related to the variations of the cultivated regions, storage conditions and maturity stage. It may also be due to geographical and climatic differences where *Nigella* seeds had been grown.

Oil content and fatty acid methyl esters profile extracted with *n*-hexane was significant with oil content, palmitic acid, oleic acid, linoleic acid, Linolenic acid, saturated fatty acid, unsaturated fatty acids, and monounsaturated fatty acid. For CM (2:1) oil content, palmitic acid, oleic acid, Linolenic acid, saturated fatty acid, unsaturated fatty acids, monounsaturated fatty acid and polyunsaturated fatty acids while with CM (1:3) stearic acid, and Linolenic acid had significant association with yield for 1st, 2nd and third years. In case of phospholipids (PG, PS, and LPE), nutritional characteristics (oil content, protein, ash, and moisture) for mineral nutrients (Ca, Cu, Pb, Zn, Cobalt, Mn, Na, P, and B were found significant association with yield based on all the three years evaluation. The results were in agreement with Maiti & Biswas (2003), who reported that grain yield plant$^{-1}$ showed a highly significant positive correlation with 100 seed weight, number of primary branches and number of capsules plant$^{-1}$ at the genotypic level. At the phenotypic level grain yield was positively correlated with all the characters except plant height. At the genotypic and phenotypic levels, grain yield exhibited the greatest correlation with 100 seed weight.

Evaluation and characterization of both phytochemicals and secondary metabolites could explore the various sources of variability and their utility at larger scale. It therefore can augment the supply not only as edible oil but also as biofuel after systematic exploitation. Moreover, these lines rich in such ingredients may comprehend the needs of the local herbal as well as pharmaceutical industry. Ali *et al*., (2012) stated that use of medicinal plants for various health disorders is a common practice especially in rural areas. While there are 2 main reasons a) poor economic condition and, b) lack of modern health care facilities in these areas are the major reasons for adopting traditional medicine. Moreover, the World Health Organization (WHO) considers that traditional medicine is an important contributor to its health goals and has been encouraging its development through testing herbs' toxicities and improving methods of herb collection, drying and conservation (Deeb *et al*., 2013).

**Table 4. The coefficient of correlation for yield with phospholipids, nutritional characteristics and mineral nutrients in 32 genotypes of *Nigella sativa* L., germplasm.**

| Phospholipids profile | Yield during | | |
|---|---|---|---|
| | 2008-09 | 2009-10 | 2010-11 |
| Phosphatidyl inositol (PI) | -0.17 | 0.04 | 0.14 |
| Phosphatidic acid (PA) | 0.11 | 0.00 | -0.01 |
| Phosphatidyl glycerol (PG) | 0.46* | 0.05 | -0.21 |
| Cardiolipins (CL) | 0.38* | -0.03 | -0.09 |
| Phosphatidyl serine (PS) | 0.10 | -0.10 | -0.24 |
| Phosphatidyl ethanolamine (PE) | 0.31* | -0.01 | -0.17 |
| Lysophosphatidyl ethanolamine (LPE) | 0.47* | 0.09 | -0.09 |
| Phosphatidyl choline (PC) | 0.02 | 0.07 | 0.17 |
| Lysophosphatidyl choline (LPC) | 0.21 | 0.04 | -0.01 |
| Sphingomyelin (SM) | 0.29 | 0.20 | -0.04 |
| **Nutritional characteristics** | | | |
| Oil contents | -0.19 | -0.06 | 0.18 |
| Protein | -0.21 | 0.33* | -0.09 |
| Carbohydrates | 0.05 | -0.35* | -0.02 |
| Ash | -0.24 | 0.07 | 0.06 |
| Moisture | 0.11 | 0.44* | -0.17 |
| Fiber | 0.19 | 0.27 | 0.01 |
| **Mineral nutrients** | | | |
| Nitrogen (%) | -0.11 | -0.01 | 0.08 |
| Iron (Fe) mg/kg | 0.09 | -0.13 | 0.00 |
| Calcium (Ca) mg/kg | 0.22 | 0.09 | 0.22 |
| Cupper (Cu) mg/kg | 0.27 | 0.17 | -0.24 |
| Magnesium (Mg) mg/kg | -0.04 | -0.01 | -0.22 |
| Lead (Pb) mg/kg | 0.36* | 0.13 | 0.15 |
| Zinc (Zn) mg/kg | 0.18 | 0.06 | -0.34* |
| Cobalt (Co) mg/kg | 0.11 | 0.24 | -0.08 |
| Magnesium (Mn) mg/kg | -0.07 | -0.15 | -0.26 |
| Sodium (Na) mg/kg | 0.10 | 0.39* | -0.15 |
| Phosphorus (P) (%) | 0.09 | -0.05 | -0.17 |
| Boron (B) ppm | -0.11 | -0.26 | 0.23 |
| Potassium (K) (%) | -0.01 | -0.33* | -0.23 |

**Highly Significant, *Significant at 0.01% and 0.05% probability level

*Nigella sativa* L., germplasm evaluated during this study indicated that it is well adopted in prevailing conditions but at the same time yield over the years also influenced by the environmental fluctuations. Although, the seeds were preserved and conserved at appropriate conditions at Plant Genetic Resources Program but the evaluation and characterization on year basis is also suggested to get healthy and fresh seeds for the sowing of up coming season. On the broader spectrum, there is no denying of its industrial importance it could become one of the cash economic crops of the Pakistan to get good returns from one acre per yield. Few medicinal herbs and spices are well adopted in our local climate but consistent negligence and unavailability of proper cultivation and post harvest processing facilities make major hindrance for their production on large scale. Expending global market and recent advances in agricultural biotechnology the medicinal plants and herbal industry also encourages small landholder farmers to earn more and sufficient from their proper selection of the herbs and spices. The evaluation and characterization of agriculturally important traits could help to develop future strategies to work on this crop with more interest and inputs.

## Conclusion

In conclusion, four genotypes viz., Pk-020545, Pk-020561, Pk-020576, and Pk-020646 indicated stability in yield over three years evaluation. Hence these are suggested to be exploited for future hybridization and breeding programs. Further, farmers with marginal lands could use these genotypes directly to get high returns.

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