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### Genetic Divergence in Cowpea (Vigna unguiculata L. Walp) - an Overview

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### ABSTRACT

Cowpea [*Vigna unguiculata* L.Walp] is an important summer/rainy season legume crop which is tolerant to drought as well as water logging conditions. It provides nutritious grain and an inexpensive source of protein. In cowpea breeding programmes, the major emphasis has been on the collection and conservation of genetic pools. The knowledge on the nature and extent of genetic variability present in any crop species plays an important role in designing a suitable breeding method. Genetic diversity is the foremost basic requirement for a successful breeding programme. Heritability is a biostatistic commonly used in plant breeding and genetics works that estimates how much variation in a phenotypic trait in a population is due to genetic variation among individual plants in that population. Genetic advance is the improvement in the mean genotypic value of selected plant families over that of base population. It depends upon phenotypic variability existing for different characters and classification into groups help in identifying potential distinct genotypes which are having contrasting characters, can be used to operate effective selection of genetically diverse genotypes for the improvement of yield. In this review paper, the research work carried on different aspects of genetic divergence is discussed under the sub-heads, i.e. variation of qualitative and quantitative traits, genetic variability, heritability and genetic advance, correlation and path analysis, genetic divergence using D<sup>2</sup> analysis and genetic diversity by using molecular markers.

Keywords: Cowpea, genetic variability and divergence, heritability, genetic advance.

### Introduction

Cowpea [Vigna unguiculata L.Walp] (2n=22) is an important summer/rainy season legume crop which is tolerant to drought as well as rain water stagnation conditions. Like other legumes, it also has unique ability to fix atmospheric nitrogen into nitrate, thus improve fertility status of soils (Kumar et al. 2015). Cowpea is a dicotyledonous crop plant in the order Fabaceae, subfamily Faboideae (Papillionoideae), tribe Phaseoleae, subtribe Phaseolinae, genus Vigna. The genus Vigna is pantropical and has high variability. In addition to cowpea, other members include mungbean (V. radiata), adzuki bean (V. angularis), blackgram (V. mugo) and the bambara groundnut (V. subterranea). Cowpea, V. unguiculata subspecies *unguiculata* includes four cultigroups: *unguiculata*, *biflora* (or *cylindrica*), *sesquipedalis* and *textilis* (Ng and Maréchal 1985). Moreover, *V. unguiculata* subspecies *dekindiana*, *stenophylla* and *tenuis* are the immediate wild progenitors of cultivated cowpea. Based on the distribution of diverse wild cowpeas along the entire length of eastern Africa, east and southern Africa was proposed to be the primary region of diversity and west and central Africa to be the secondary center of diversity (Steele 1972; Singh et al. 1997; Timko and Singh 2008; Lal and Vashisht 2008; Rana, 2011). These authors also suggested Asia as a third center of diversity, specific for two cultigroups *cylindrical* (catjang bean) and *sesquipedalis* (yardlong bean).

Cowpea provides nutritious grain and an inexpensive source of protein for both rural poor and urban consumers and it is also called as vegetable meat (Lal and Vashisht 2008; Ram 2014). Cowpea grain contains about 23-28% protein and 64% carbohydrate and therefore, has an enormous potential to contribute to the alleviation of malnutrition among resource-poor farmers (Bressani 1985; Singh et al. 1997; Lal and Vashisht 2008). Besides being used as pulse in form of dry seed, cowpea's immature pod and green leaf and growing twig can be utilized as vegetable. However, it is more important as the source of green as well as dry fodder. Among fodder legumes, cowpea is grown for both grain and fodder in all tropical and sub-tropical climates. Area, production and yield under cowpea cultivation for dry grain over the world were 12.6 million hectares, 5.6 million tonnes and 443 kg/ha, respectively. In which Niger, Nigeria, Burkina Faso are the first rankings in harvested area and production also (FAOSTAT, 2014). In India, cowpea is grown as sole, inter-crop, mix-crop and in agro-forestry combinations. Its area is estimated to be cultivated in almost half of 1.3 m ha of area occupied by Asian region. Cowpea is a minor pulse in Indian cultivated mainly in arid and semi-arid tracts of Rajasthan, Karnataka, Kerala, Tamilnadu, Maharashtra and Gujarat. In northern India, it is mainly grown for fodder during *kharif* as well as summer in pockets of Punjab, Haryana, Delhi and West UP along with considerable area in Rajasthan (Tiwari and Shivhare 2016).

In cowpea breeding programmes, the major emphasis has been on the collection and conservation of genetic pools. Cowpea germplasm is maintained in collections around the world with varying levels of accessibility and documentation. The largest collections are held by the IITA over 16000 cultivated varieties and almost 2000 wild accessions of cowpea and some other collection from the United States Department of Agriculture (USDA), University of California-Riverside, India, Brazil, these collections that cover a wide spectrum of growth habits, environmental responses and varying pest and disease susceptibilities (Singh et al. 1997; Timko and Singh 2008; IITA 2017). The knowledge on the nature and extent of genetic variability present in any crop species plays an important role in designing a suitable breeding method. Genetic diversity is the foremost basic requirement for a successful breeding programme. A quantitative assessment of the genetic divergence among the collection of germplasm and their relative contribution of different traits towards the genetic divergence provide essential and effective information to breeder in his hybridization programme and thereby genetic improvement of yield (Nagalakshmi et al. 2010).

It is difficult to judge what proportion of observed variability is heritable and non-heritable i.e. environmental. The breeding in cowpea is mainly depends on magnitude and nature of interactions of genotypic and environmental variations. It is imperative to partition the observed variability into its heritable and non-heritable components and to have an understanding of parameters like genetic coefficient of variation, heritability and genetic advance (Thakur et al. 2011). Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) are measured to study the variability presenting in the germplasms. The high GCV and PCV encompass the possibility of large variation. Therefore, its correct understanding is very important for efficient utilization in crop improvement programme. Sivasubramanian and Madhavamenon (1973) classified GCV, PCV into three groups as: 0-10% = 10w; 10-20% = moderate; >20% = high.

Knowledge of heritability influences the choice of selection procedures, to predict gain from selection and to determine the relative importance of genetic effects. Evaluations of the components of variation and heritability are, therefore, among traits that will facilitate improvement of crops such as cowpea. Genetic advance is the improvement in the mean genotypic value of selected plants over the base population. It depends upon phenotypic variability, heritability and intensity of selection (Ngoc et al. 2019). When, genetic advance as percent of mean (GAM) was high for the trait, effective progress in improvement through selection could be achieved in cowpea (Ajayi et al. 2014). Correlation among traits also helps to simultaneously select for more than one trait of importance at a time. Yield is a complex character determined by several component characters. Therefore, selection for yield should take into account related traits as well. Path coefficient analysis provides an effective means of partitioning the genotypic correlation coefficients into direct and indirect effects of the component traits on yield on the basis of which crop improvement programmes can be logically devised (Vidya and Oommen 2002).

In cowpea, quantification of the magnitude of variability in characters and classification into groups help in identifying potential distinct genotypes having contrasting characters can be used for effective selection of genetically diverse genotypes for yield improvement. Keeping these aspects in view, the proposed review was undertaken the following subheads:



### Variation of qualitative and quantitative traits

Traditionally, genetic diversity evaluated in crop species are based on differences in morphological characters and qualitative traits (Kumar et al. 2015), probably due to the fact that the assay of qualitative traits does not need any sophisticated equipment or complex experiments, they are generally simple, rapid and inexpensive to score. It has been used as a powerful tool in the classification of cultivars and also to study taxonomic status. It is this precious source of material that serves as the essential foundation for the breeding of new improved varieties. Such characteristics are often controlled by multiple genes and are subject to varying degrees of environmental modification and interaction. Qualitative traits, such as yield performance and quality characters are of major importance in breeding and consequently, these traits are usually focused on during the evaluation of accessions. However, these traits express high environmental effects and often also genotype with environment interaction. Tanksley et al. (1989) emphasised the fact that many of the morphological traits are also difficult to analyse because they do not have the simple genetic control assumed by many in genetic models. Magloire (2005) studied 20 cowpea accessions from Cameroon, South Africa, Kenya to assess agro-morphologic diversity among them. Based on 15 qualitative traits (growth pattern, twinning tendency, stem pigmentation,...) and 12 quantitative traits (plant height, pod length, 100 seed weight,...) which resulted in high genetic variability in almost these traits.

Muhammad et al. (2010) compared four local fodder cowpea genotypes namely CP-1, CP-8, CP-31 and CP-801 at Peshawar. CP-1 required minimum days to emergence (4.7 days), flowering (87 days) and pod formation (94.7 days) and the shortest plant length (235 cm). CP-8 produced the longest plants (382 cm) though statistically not different than CP-31 and CP-801. CP-8 gave the maximum grain yield (9691 kg/ha), seeds/pod (11.3) and 100 seed weight (11.4 g). Generally, the fresh fodder yield in all germplasm gradually increased from 14 ton/ha, 50 days after emergence to 64.2 ton/ha, 125 days after emergence. They concluded that maximum fresh fodder can be obtained 125 days after emergence and CP-8 gave the maximum grains as well as fresh fodder yield among the germplasms tested.

Kumar et al. (2015) carried out the characterization of 20 forage cowpea genotypes. All genotypes showed indeterminate type of main stem and raceme type of layer in canopy; whereas genotypes *viz.*, KBC 2, IC 249141, HC 46, CO 4, EC 3941-1, EC 101980, Kohinoor and CO 5 showed unique state of expression among nine characters viz., incidence of cowpea mosaic virus, seed crowding in pod, text a structure, eye colour, pod shape, pod attachment to peduncle, terminal leaflet shape, twining tendency and flower pigmentation, respectively. While two genotypes HC 46 and EC 3941-1 had wrinkled text a structure with pigmented wings and tan (brown) eye colour with curved pod shape, respectively. Remaining genotypes showed various states of expression in a group of two or more genotypes together. Animasaun et al. (2015) investigated ten cultivars of cowpea in the screen house in Nigeria. The results showed considerable variations in growth and yield characters evaluated. Cultivars NGB/06/047, IFE BROWN 2012 and IT98K-133-1-1 had optimal growth performance with respect to fruiting and seed yield parameters. Proximate results indicated that crude protein varied from 23.42-26.78%. Ash content ranged between 3.60-4.21%, crude fibre varied from 2.10-2.98% and carbohydrates 56.10-59.59%.

Nguyen et al. (2016) carried out an experiment on 30 genotypes of cowpea. Both within and between group variations was studied. The group low foddermedium grain yield had maximum genotypes i.e. RC-101, GC-13-1, TC-142, TC-141, PGCP-11, KBC-5, GC-5, Pant lobia-3, while CP-05040, GPCP-28, PGCP-24 and GC-1105 having considerable value for grain yield. However, in low fodder-high grain yield group only one genotype *i.e.* PGCP-2-3 was placed. However, only three genotypes, viz. KBC-5, Goa Cowpea-3 and GC-901 were included in the high fodder-low grain yield production and could be utilized for cowpea fodder production after testing over time and space. The genotype TPTC-29 was included in medium fodder-medium grain yield and one genotype (GC-1106) was included in medium fodder-high grain yield, which had excellent grain production as well as good potential for fodder production. It could be utilized for dual purpose after extensive testing over time and space.

Vu et al. (2017) recorded the maximum seed yield per plant by genotype PL-3 (49.63g), followed by CPD 240 (46.66), RC 101 (42.78), KBC 10 (36.11) and PGCP 28 (35.56) which will be most promising to exploit and utilize for incorporation of yield potential. Some other genotypes having high mean value for other characters can be used as a donor. The highest biological yield per plant was observed in genotype UCP 12-007 (443.89g), followed by KBC-6 (426.67), KBC-8 (392.89), GC 901 (377.22) and VCP 09-019 (332.78). Being utility of cowpea as fodder crop high biological yield is needed. With respect to seed protein content, the mean seed protein content was exhibited highest (26.69%) in the genotype TPTC-29. Besides, the genotypes PCP-07-272, Goa Cowpea-3, UCP 12-007 and VCP 09-019 also indicated high seed protein content (26.40, 25.81, 25.38 and 25.08%), respectively. More or less similar findings were also supported by (Lazaridi et al. 2017).

### Genetic variability, heritability and genetic advance

The presence of considerable amount of variability in the basic genetic material ensures better chances of evolving desired plant types. The estimates of heritability, PCV, GCV and genetic advance are helpful to determine the method of selection to improve a particular plant population for a specific trait. The magnitude and type of genetic variability help the breeder to determine the selection criteria and breeding schemes to be used for improvement purposes (Omoigui et al. 2006). Ariyo (1995) evaluated twentysix varieties of cowpea to study genetic variability, inter-character relationships as well as the path analysis of components of seed yield. Seed yield was the most variable character with GCV and PCV of 22.11 and 35.25%, respectively. Weight of 100 seeds had the highest heritability estimate of 92.33% while number of peduncles per plant exhibited the lowest heritability estimate of 30.11%.

Manonmani et al. (2000) studied ten genetically diverse genotypes of cowpea recorded highest GCV (29.12), PCV (31.13) and GA (56.15) values with 87.5%  $h^2$  for FY, whereas the highest  $h^2$  (96.9%) was observes in dry fodder. Vidya et al. (2002) evaluated 50 varieties of yard-long bean were for yield and a few related characters. High genotypic coefficient of variation, heritability in broad sense and genetic advance estimated for the characters viz., yield of vegetable pods per plant, number of pods per plant and pod weight indicated the scope for improvement of these characters through selection. Chauhan et al. (2003) studied the genetic parameters in 18 forage cowpea genotypes. Genotypic and phenotypic coefficients of variation, broad sense heritability and genetic advance of yield components, *i.e.* days to maturity, plant height, pods per plant, pod length, seeds per pod, 100-seed weight, plant stand, seed yield per plant and seed yield per plot. The additive gene effects were significant for plant height, pods per plant, plant stand and 100-seed weight.

Lesly (2005) carried out evaluation 169 genotypes which showed the high PCV and GCV values were recorded by seed yield per plant, hundred seed weight, harvest index, number of pods per plant and germination percentage. Both GCV and PCV values showed similar pattern of changing over the characters. All the characters showed high heritability except



seeds per pod, pod length and number of branches per plant. The highest heritability was recorded by hundred seed weight. High genetic advance was observed for germination percentage, plant height, number of cluster per plant, number of pods per plant, hundred seed weight, harvest index and seed yield per plant. Malarvizhi et al. (2005) studied 60 genotypes for genetic variability, heritability and genetic advance of fodder cowpea. All the 60 genotypes varied significantly for all the 13 characters. They reported high heritability and genetic advance for number of branches per plant, number of leaves per plant, dry weight of leaves, dry weight of stem, dry matter yield and plant height, indicating that these traits were controlled by additive genetic effects, providing better source population for developing high-yielding fodder cowpea cultivars.

Omoigui et al. (2006) carried out a screen house experiment at Samaru, Nigeria in 1999 and 2000 dry seasons to estimate the genotypic variability of some reproductive traits and their heritability in nine cowpea varieties. Results of the study showed that there was considerable variation among cultivars for duration of reproductive phase and rate of photosynthate partitioning. Genotypic coefficients of variation were also high for days to first flower, 100-seed weight, plant height and harvest index. Broad-sense heritability estimate (h<sup>2</sup>) was 98.9% for 100-seed weight, 94% for duration of reproductive phase, 84.5% for days to first flower, 83.9% for days to maturity and 77.3% for harvest index. This information showed that there is sufficient genetic variance to warrant selection for improvement in the cowpea genotypes studied.

In a study, 14 promising vegetable purpose cowpea genotypes were evaluated for fodder yield, green pod yield and other component traits. Results showed that there was considerable variation among cultivars for all the traits. Genotypic and phenotypic coefficients of variation were high for green pod yield and green forage yield. High heritability coupled with high genetic advance as per cent of mean was noticed for characters plant height, number of branches, number of pods per plant, green pod yield and green forage yield. They concluded that there was sufficient genetic variance to warrant selection for improvement in these cowpea genotypes and that considerable progress in forage cowpea breeding could be achieved by exploiting these traits (Lohithaswa et al. 2009). Idahosa et al. (2010) studied genotypic variability of eight parent line cowpeas to evaluate some genetic parameters namely coefficient of variation, genetic variance and heritability estimates in the broad-sense. Per se mean performance was variable among the genotypes for all characters investigated which indicated the superiority of some parent lines. Highly significant heritability effects were observed for all characters except for 100-seed weight (42.2%) which expressed moderate heritability estimate. Days to 50% flowering, pod length, pod weight and grain yield characters showed that some levels of genetic variability existed.

Two genetically diverse parents belonging to determinate and indeterminate were crossed (V-1188 x Goa local) and advanced to F<sub>2</sub> and F<sub>3</sub> generations. These were evaluation for estimation genetic variability in various characters viz. plant height, branches per plant, canopy spread, clusters/plant, pods/plant, seed per pod, pod length and 100 seed weight, seed yield. There were differences in GCV and PCV values in both F<sub>2</sub> and F<sub>3</sub> population and GCV, PCV were moderate to high in  $F_2$  comparing low to moderate in  $F_2$ . Similar trend was also observed with heritability and genetic advance as per cent mean that have declined in F<sub>3</sub>. Plant height, pods/plant, seed yield could be considered for selection as they had higher PCV and GCV values coupled with appreciable heritability and genetic advance as per cent mean (Shashidhar et al. 2010).

Crosses were made between five cultivated cowpea varieties and a wild relative var. pubescens (as pollen parent) to study the inheritance of hairiness, pod shattering, as well as heritability and correlations among yield and yield related traits. F<sub>1</sub> plants exhibited dominance for both hairiness and pod shattering traits. Broadsense heritability estimates showed that days to pod maturity averaged 77.93%, 100 seed weight 68.45%, seeds per pod 69.76% and number of branches per plant had 62.54% (all high). Days to first flowering and pod length were moderate (57.31 and 54.29%, respectively). Number of pods per plant had low heritability estimates with an average of 39.0%. High broad sense heritability averaged 77.93% for days to pod maturity, 68.45% for 100 seed weight, 69.76% for seeds per pod and 62.54% for number of branches per plant. Days to flowering and pod length per plant had moderate estimates of 57.31 and 54.29%, respectively (Mohammed et al. 2010).

Singh et al. (2010) observed significant mean square for all the traits indicating adequate variability among the genotypes. High phenotypic and genotypic coefficient of variation coupled with high heritability and genetic advance as per cent of mean were reported for plant height, stem weight, leaves weight, biological yield, dry matter yield and green fodder productivity indicating predominance of additive gene effects in controlling these characters. Green fodder yield was significantly and positively correlated with green fodder productivity, dry matter yield, biological yield, leaves weight and stem weight. Path coefficient analysis revealed that the stem weight had maximum and positive direct effect on green fodder yield at phenotypic and genotypic level followed by leaves weight and dry matter yield. Biological yield and dry matter per cent had negative direct effect on green fodder yield.

In a study of 10 cowpea accessions, (Manggoel et al. 2012) revealed PCV and GCV were high for the trait's days to 50% flowering, number of flowers/plant, number of peduncles/plant, number of pods/plant, 100-seed weight and grain yield (26.54-41.46), except pod length and seeds per pod. High broad-sense heritability was high (63.16 - 96.74%) for all these characters. Nwofia et al. (2012) investigated twelve genotypes of cowpea in two seasons 2010 and 2011. It was found that plant height, number of branches, leaves, pods and dry matter per plant, number of seeds/pod and seed weight/pod, pod length gave moderate to high at both GCV and PCV. Heritability and genetic variance of all these characters were also high 54.0-94.95 and 12.89-99.01, respectively.

Costa et al. (2013) studied 57 African cowpea lines from the Cowpea Germplasm Bank of Embrapa Meio-Norte, Brazil to estimate the genetic divergence between cowpea lines. Genotypic coefficient of variation was highest for the traits yield (19.77%) and 100 seed weight (16.33%) and lowest in grain length (8.95) and grain width (7.48). The heritability estimates ranged between 37.38 and 95.92% for NPP and W100G, respectively. The heritability values of the traits PL, NGP, GRL and W100G were all higher than 70%, indicating the possibility of genetic progress with selection.

Ten genotypes of cowpea were studied for the interrelationship among quantitative traits. Estimates of phenotypic and genotypic coefficients of variation, broad sense heritability and genetic advance as percent of mean and correlations were performed on 20 quantitative traits. Genotypes differed significantly for all traits studied which showed the existence of sufficient genetic variability among the tested genotypes. High broad sense heritability values for all traits studied except for plant height (moderate) shows that these traits are less influenced by environmental effects. The high positive genotypic and phenotypic correlations between numbers of pods per plant, number of seeds per pod, number of seeds per plant and seed weight indicates that selection for these will result in increase in yield (Ajayi et al. 2014).

In a study, 17 genotypes of vegetable cowpea (*V. unguiculata* cv-gr. *sesquipedalis*) collected from different places in the country were evaluated for different horticultural traits. Genetic variation, character

association, cause-effect analysis and genetic diversity was measured among the genotypes. Chattopadhyay et al. (2014) found out all the eight characters under study differed significantly among the genotypes. High to moderate GCV and PCV values were found for number of pods per plant, pod yield per plant, pod weight, number of seeds per pod and pod length. The PCV agreed closely with GCV for all the characters except pod width and pod yield per plant (the GCV ranged from 10.29% to 32.35%, while PCV ranged from 10.40% to 32.93%). Higher estimates of broad sense heritability coupled with higher genetic advance for number of pods per plant, pod yield per plant, pod weight, number of seeds per pod and pod length.

Santos et al. (2014) studied 20 genotypes which also revealed coefficients of heritability based on the mean of families showed magnitudes from moderate to high for the character of days to flowering, green pod length, number of beans per pod, number of pods per plant and hundred seed weight, confirming that most of the phenotype is attributed to genetic causes. The low value of heritability and the high value of the phenotypic coefficient of variation observed for grain yield. Gerrano et al. (2015) estimated the level of phenotypic variability among a collection of 25 cowpea genotypes in South Africa. Sixteen phenotypic markers were recorded. the differences among genotypes were highly significant for all traits. This indicated the high level of genetic variability among the cowpea genotypes studied. Genetic and phenotypic coefficient of variation and broad-sense heritability were estimated for all phenotypic traits.

Khanpara et al. (2015) observed significant differences among the genotypes for all the 12 characters studied. The highest range of variation was observed for green pod yield per plant followed by ten pod weight, number of pods per plant and plant height. Higher values of PCV than GCV were observed for green pod yield per plant, number of pods per plant, pod length and number of seeds per pod which indicates partly interaction of the genotypes with the environment or other environmental factors influencing the expression of these characters. The value of PCV and GCV more or less equal were observed in the remaining characters which indicated that these characters were less influenced by the environment. High heritability along with high genetic advance was observed for green pod yield per plant, plant height, pod length, pod width, number of seeds per pod, number of pods per plant, ten pod weight, number of pods per cluster and hundred fresh seed weight indicating that these traits were mainly governed by additive gene action and responsive for further improvement of these traits.



The investigation was carried out on 15 genotypes to study genetic parameters for ten quantitative characters of bush type cowpea. The differences among all the genotypes were statistically significant for all the ten quantative characters. High estimates of genotypic coefficient variation, heritability (broad sense) and genetic advance were observed for the characters such as plant height, number of pods per plant, edible pod yield per plant as well as edible pod yield per hectare, suggesting additive gene action for expression of these characters (Tudu et al. 2015). Genetic variance components indicate high genetic contributions over non-genetic to plant phenotypic variability with high heritability values (0.75-0.91) by investigation of Aliyu and Makinde (2016).

Chandrakar et al. (2016) studied 21 genotypes of vegetable cowpea. They found highly significant differences among the genotypes for all the characters except pod width. It indicates that sufficient variability existed among the genotype. The GCV value was higher for plant height, pod yield per plant indicated that these characters exhibited variability among the genotypes. The highest heritability was recorded for the characters plant height (60 DAS), days of maturity, fruiting duration, 50 per cent flowering, pod setting per cent per plant, 100 dry seed weight, 100-green seed weight. Khandait et al. (2016) reported the highest PCV and GCV for characters viz., number of flower cluster per plant, number of pods per plant, number of pods cluster, number of branches at 30 DAS, pod weight and pod length. Heritability estimates were observed very high for pod length, number of pods per plant, pod weight, number of flower cluster per plant and pod width. The highest estimate of genetic advance as percentage of mean was recorded for number of flower cluster plant followed by number of pods per plant, pod length, number of pods per cluster, pod weight, pod width, number of flowers per cluster, number of branches at 30 DAS, pod yield plot, pod yield per ha and pod yield per plant.

Sixty-six bush type advance breeding lines of vegetable cowpea developed at Varanasi were evaluated for different horticultural traits for genetic variability, character association, cause effect analysis and genetic diversity among the genotypes through D<sup>2</sup> statistics. High values of genotypic and phenotypic coefficient of variations, heritability (h<sup>2</sup>B) and genetic advance were recorded for pod yield per plant, number of peduncles and pods per plant, peduncle length, number of primary branches per plant, pod length, pod weight and number of seeds per pod (Lal et al. 2017). Magashi et al. (2017) studied genetic variability for growth and yield in cowpea varieties. They found highly significant

difference in the plant height, number of days to 50% flowering, number of days to maturity, number of pods per plant, pod length, number of seeds per plant, 100 seed weight ash and protein content. While significant difference was found seedling height, number of branches per plant and fibre content. No significant difference was found in the remaining.

Genetic variability, heritability, genetic advance and genetic advance as per cent of mean for twelve characters were assessed by field evaluation of 22 genotypes at Vellanikkara. The high degree of variability was observed for all the characters. High magnitude of the PCV, GCV, heritability and genetic advance was observed for plant height, grain yield per plant and length of the pods. Seeds per pod and protein content exhibited low PCV and GCV, but high irritability and low genetic gain. The difference between the phenotype coefficient of variation and genotype coefficient of variation were found maximum in pod weight (30.15%), followed by number of pods per plant (18.12%) and test weight (16.27%), (Sarath and Reshma, 2017).

The magnitude of phenotypic coefficient of variance was slightly higher than genotypic coefficient of variance for all the characters, which revealed the less sensibility of characters to environmental factors under field conditions. The high estimates of GCV and PCV was observed for biological yield per plant, followed by plant height, harvest index, number of clusters per plant, pod yield per plant, seed yield per plant, number of pods per plant, number of branches per plant, pod weight and days to 50% flowering (Ngoc et al. 2019).

The estimates of heritability (broad sense) were observed high in biological yield per plant, followed by plant height, number of clusters per plant, days to 50% flowering, days to maturity, pod weight, harvest index, seed yield per plant etc. Further, genetic advance as per cent of mean observed high for characters like biological yield per plant, followed by plant height, harvest index, number of clusters per plan, seed yield per plant, pod yield per plant etc., while the estimates of genetic advance as per cent of mean was moderate for pod breadth and number of pods per cluster indicating that the improvement of these through selection as well as their exploitation through combination breeding. The estimates of high heritability coupled with high genetic advance, suggesting that simple selection could be done for the improvement of these traits (Ngoc et al. 2019).

High heritability values in yield contributing the characters are useful while making selection, it indicated that the variation in these characters was mainly under genetic control and was less influenced by environment but selection based on this factor alone may limit the progress, as the same very prone to environmental changes (Maurya et al. 2014). It is apparent that the improvement of various characters, individually or simultaneously, different selection intensities are to be exercised depending on estimates of genetic components of variability in view of their heritability. High genetic variations combined with high heritability could provide effective selection of phenotypic trait for further improvement in cowpea through hybridization. The results in genetic analysis of triple test cross by Mittal et al. (2010) also indicated the additive genetic variance was presented for most of characters and suggested simple selection to get improvement in cowpea. The evaluation of cowpea genotypes may be reliable based on study of heritability; however, still more solid base may be formed by estimating the performance through genetic advance. Johnson et al. (1955) stated that heritability estimated coupled with genetic advance were more helpful than heritability alone in predicting the progress from the selected better genotypes. However, there are limitations of using broad sense heritability as it includes both additive and non-additive gene effects. Therefore, it is necessary to estimate broad sense heritability in conjunction with the genetic advance.

#### Correlation and path analysis

The success of cowpea as a pulse crop largely depends on the extent of improvement made for seed yield. Selection is based on the performance of grain yield a polygenically controlled complex trait is usually not very efficient. But the one based on its component character could be more efficient. The study of association of seed yield with other component characters helps the breeder to select for the characters and to construct a suitable plant type leading to higher seed yield. The importance of correlation studies in selection programmes is appreciable when highly heritable characters are associated with the important character like yield. Path coefficient is an excellent means of studying direct and indirect effects of interrelated components of a complex trait particularly if the high correlation between two traits is a consequence of the indirect effect of other traits. Path-coefficient analysis measures the direct influence of one variable on another. By determining the interrelationships among grain yield components, a better understanding of both the direct and indirect effects of the specific components can be attained.

The experiment by Ariyo (1995) showed that number of peduncles per plant, number of branches per plant showed significant and positive genotypic and phenotypic correlation with seed yield. The number

of pod per peduncle only showed positive genotypic correlation with seed yield. Path coefficient analysis revealed that seed yield was affected by weight of 100 seeds, number of seeds per pod, number of branches per plant, number of peduncles per plant and number of pods per peduncle. Kumar et al. (2001) studied 72 diverse genotypes of cowpea to find out correlations and path analysis for grain yield and its related eight traits. Genotypic correlations were higher than their corresponding phenotypic correlations. The grain yield per plant was positively correlated with all the traits except days to maturity and seeds per pod. 100-seed weight had the maximum direct and indirect effect on grain yield of cowpea. Most of the component traits had positive association among themselves and with grain yield of cowpea.

A total of 600 cowpea germplasm lines were evaluated in augmented randomized block design. A wide spectrum of variability was observed for all the characteristics except for days to maturity and number of seeds per pod which exhibited moderate variability. Seed yield showed positive correlation with 100-seed weight and pod length. While positive association of 100-seed weight with pod length and plant height revealed that taller plants, in general, had longer pods with bold seeds resulting in higher seed yield (Singh and Verma 2002).

In a study, eight cowpea genotypes and cultivars from different locations in Turkey were evaluated association analysis. Pod length and 100 seed weight showed a positive and highly significant correlation with seed yield per plant. Path analysis results revealed that pod length had the highest direct positive effect on seed yield per plant, followed by 100 seed weight and pods number per plant. On the other hand, seed yield per plant was directly and negative affected by first pod height and branches number per plant (Peksen and Artik 2004).

Lesly's evaluation (2005) resulted in number of clusters per plant, number of pods per plant, harvest index and hundred seed weight were significantly correlated with seed yield both at genotypic and phenotypic levels. Number of clusters per plant and pods per plant significantly associated with harvest index both at genotypic and phenotypic levels. The highest direct effect recorded by harvest index and higher indirect effect of number of clusters per plant and number of pods per plant through harvest index was exhibited at genotypic and phenotypic level. Kumari et al. (2005) evaluated 50 genotypes of cowpea grown during *kharif* 2001 at Hisar for tannin content, crude protein and in vitro protein digestibility. Crude protein ranged from 27.57-21.15%. The in vitro protein



Mittal et al. (2006) observed that forage yield in cowpea can be improved by selecting plants with greater stem diameter and length in the segregating populations of the cross at PAU, Ludhiana. Bhandri and Verma (2007) revealed that plant height, number of leaves per plant, leaf: stem ratio, crude protein and dry matter digestibility had major contribution in determining quantity and quality of green and dry forage yield. Ajeigbe et al. (2008) reported that variations in protein content among cowpea varieties. High positive correlations (0.86) were observed between the content of fat and crude fibre, ash and protein (0.78), carbohydrate and viscosity of cowpea flour (0.76) and between ash and tannin (0.61) content of cowpea seed, negative correlations were observed between the content of crude protein and carbohydrate (-0.98) in cowpea seed. The physicochemical properties evaluated generally had high broad sense heritability (56-99%).

In study of Mohammed et al. (2010), correlations among number of pods per plant, seeds per pod, seeds per plant and seed yield per plant (0.485-0.568) were significantly positive. Correlations between seed per pod and seed per plant with number of pods per plant were 0.909 and 0.996, respectively. Umar et al. (2010) studied the morphotypic variations of eight local varieties of cowpea in relation to their yield in 2004 rainy season. The magnitude of the genotypic correlation coefficients was in most cases higher than their corresponding phenotypic (rp) and environmental (re) correlation coefficients. High and positive rg exists between days to 50% flowering and plant height (rg=0.9113), days to maturity and fodder weight (rg=0.9301), days to maturity and 100 seed weight (rg =0.6958) and number of leaves per plant and fodder weight (rg=0.8096). On the other hand, high but negative rg exists between plant height and pod per plant (rg=-0.6011). Also, the relationship between days to maturity and number of seed per pod were all negative and moderate.

The experiment by Ushakumari et al. (2010) using fourteen genotypes of cowpea in India to study the nature of genotypic association between the eight yield contributing characters and direct and indirect effects of the different characters on yield. The study revealed that the days to maturity, number of branches per plant and number of pods per plant showed positive significant



correlation with seed yield. Path coefficient analysis exhibited days to fifty per cent flowering, plant height, pods per plant showed positive direct effect on yield except days to maturity, branches per plant, seeds per pod and hundred seed weight which showed negative direct effects. The characters plant stands at harvest and pods per plant exhibited moderate direct effects on yield. The implications of the results revealed that selection can be done for days to maturity, number of branches per plant and number of pods per plant for improving the grain yield in cowpea.

Carvalho et al. (2012) carried out evaluation on eight parent cowpea lines/cultivars and twenty eight combinations  $(F_2)$  derived from these parents to find out the association between quantitative traits and yield. Results have shown that genotypes with high pod weight and pod length, 100-grain weight and number of beans per pod should be used to improve grain yield in cowpea. In the study of Manggoel et al. (2012) found out positive correlation between grain yield and number of peduncles per plant, flowers per plant, pods per plant and 100 seed weight. Path analysis showed high positive direct effects of number of peduncles plant, flowers per plant and 100 seed weight. Seed yield was found positively and significantly correlated to number of leaves per plant, seed weight per pod, dry matter yield per plant in 2010 and number of pods per plant in 2011. The consistent positive direct effect of number of leaves on yield in both years and high magnitude direct effect of seed weight in 2010 and number of pods per plant in 2011 suggests that the traits have high direct positive influence on seed yield (Nwofia et al. 2012).

Results trial on 4 local varieties by Udensi et al. (2012) revealed that significant relationships between yield and yield-contributing traits existed which could be indices for selection. Genotypic correlations coefficients were high and more significant than the phenotypic and environmental correlation coefficients. Path coefficient analysis shows that number of pod per plant had the highest direct effects to cowpea yield. This was followed by number of flowers, number of seeds per pod, leaf area at 5 weeks and pods length and 100 seed weight, respectively. Other morphological traits had negative direct effects on seed yield such as vain length at 10 weeks, number of leaves at 5 weeks, number of leaves at 10 weeks, days to 50% flowering and days to maturity.

Sahai et al. (2013) evaluated 168 exotic and indigenous cowpea germplasm lines and four checks/ controls in augmented design to study estimates of the correlation coefficients and path analysis of morphological as well as fodder and grain yield attributes. The present study showed a high impact of direct effects of correlation and suggested that going for plant types with higher biomass per plant, dry weight per plant, stem girth, number of secondary branches, leaves per plant, pods per plant and pod clusters per plant would be effective for improving both fodder and seed yield in cowpea. Trial outcome of Ajayi et al. (2014) has given the interrelationship among quantitative traits. The high positive genotypic and phenotypic correlations between numbers of pods per plant, number of seeds per pod, number of seeds per plant and seed weight indicates that selection for these will result in increase in yield.

Association studies revealed that genotypic correlation coefficients were higher than their phenotypic correlation coefficients in most of the cases. Positive and significant phenotypic correlations were observed for number of pods per plant (r = 0.577) and number of seeds per pod (r = 0.575) with pod yield per plant. On the other hand, significantly negative correlations were exhibited for days to 1st flowering (r = -0.695) and days to 50% flowering (r = -0.660)with pod yield per plant imply that a lot of breeding programmes are needed to improve such traits. The number of pods per plant followed by pod weight showed highly positive direct effects on pod yield per plant. From the correlation and path analysis, it can be concluded that emphasis should be given on number of pods per plant and pod weight for selecting high yielding genotypes (Chattopadhyay et al. 2014).

A field trial was conducted in 2011 rainy season to evaluate the performance of five cowpea genotypes and a local cultivar at Mubi in the Northern Guinea Savanna ecological zone of Nigeria. The path coefficient analysis of grain yield and yield attributes showed that number of pods per plant gave the highest percentage yield contribution of 31.85%. This was followed by plant height at 6 WAS which contributed 5.37%. The highest combined contribution of 8.66% came from pod number and 100-grain weight. Residual percentage contribution was 49.24%. This showed that yield attributes in this study explained 50.76% of the variability in grain yield in the experimental material Furthermore, the investigation suggests that number of pods per plant, plant height and 100 seeds weight can be considered as selection criteria in cowpea (Kwaga 2014).

Investigation's result of Santos et al. (2014) also found that the greatest positive correlations between the character pairs; days to flowering x days to maturity; days to maturity x pod weight; days to maturity x number of beans per pod; pod weight x number of beans per pod; green pod length x pod weight; and number of pods per plant x grain yield. Only the variables of days to maturity and number of beans per

pod correlated in a negative and significant way with grain yield. Considering the direct positive effects on grain yield, green pod length was the variable that showed the greatest effect (1.8128). Nevertheless, total correlation of green pod length exhibited a low value (0.0847) due to the indirect negative effect of the other variables. Total positive correlation of 0.7982 was observed on the variable of number of pods per plant and grain yield, but its direct effect was negative (-0.7521), while for this variable, the indirect effect of the other variables, mainly of the number of pods per plant (1.8946), is important since it indicates the direction of correlation. The days to maturity presented direct positive effect; nevertheless, total correlation was negative (-0.3952). The days to flowering obtained high direct effect on grain yield, while the indirect effects of the other variables ratified the total correlation, showing low association with grain yield (0.1959). The variable of number of beans per pod obtained direct negative effect (-3.5249) with the variable of grain yield. The result also gave a low residual effect (0.2081).

In another study, Sapara and Javia (2014) estimated genotypic and phenotypic correlations of green pod yield with different components from 40 genotypes of vegetable cowpea. The genotypic and phenotypic correlations agreed closely with each other. Yield contributing character number of pods per plant had positive and highly significant association with green pod yield per plant at phenotypic level. Phenotypic interrelationship between days to 50% flowering and days to 1<sup>st</sup> pod picking was negatively significant with green pod yield. The genotypic and phenotypic path analysis revealed the high to moderate direct effect of green pod yield per plant with number of pods per plant and pod length. Therefore, number of pods per plant and pod length was important component for improving green pod yield in vegetable cowpea.

Seed yield of 49 accessions was studied by Shanko et al. (2014) exhibited positive and significant correlation with number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per pod and plant height. Path analysis revealed that, yield per plant had the maximum positive direct effect on seed yield followed by number of pods per plant, while number of secondary branches per plant, days to flowering, days to maturity and number of seed per pod exhibited negative direct effect phenotypically. In addition, genotypic path analysis revealed that, maximum direct effect on seed yield was exerted by number of pods per plant and yield per plant. However, days to 50% flowering, days to maturity, number of secondary branches per plant and number of seed per pod exerted negative direct effect on seed yield.

Meena et al. (2015) evaluated 72 cowpea germplasms which resulted in seed yield per plant had positive significant correlation with days to 50% flowering, plant height, primary branches per plant, pods per plant, pod length, seeds per pod and 100-seed weight at both genotypic and phenotypic levels. Path coefficient analysis revealed that primary branches per plant and 100-seed weight had high direct positive effect on seed yield per plant at both genotypic and phenotypic levels. Pod length, days to maturity, 100 seed weight and pod wall proportion had high positive direct effects on seed yield per plant at only genotypic level. From the correlation studies on 15 genotypes, Tudu et al. (2015) found that there was a good deal of association among yield and its various attributes. Number of branches per plant, edible pod yield per plant and number of seeds per plant are important correlated characters contributing towards pod yield in bush type cow pea.

Relationships between flowering; pod maturity and seed size were positive and significant. By contrast, pods per plant, seeds per plant and total seed yield recorded negative correlations with pod maturity. However, seeds per plant and pods per plant are the most contributory components to seed-yield with correlation coefficients of r=0.95, 0.89, respectively. Although seed size had negative correlation with seeds per pod, but strong linkage between seed number (seeds per pod, seeds per plant) and seed yield (Aliyu, and Makinde 2016). The investigation of Patel et al. (2016) was carried out on 32 diverse genotypes of cowpea to study the correlations and path coefficient for different traits. Association analysis between green pod yield per plant and other eleven quantitative characters revealed that green pod yield per plant was highly significant and positively correlated with pod length and sugar content. Path coefficient analysis indicate the highest positive direct effect on green pod yield per plant by pod length followed by days to 50 per cent flowering, shelling %, number of pods per plant, sugar content and plant height at final harvest. Lal et al. (2017) investigated sixty-six bush type advance breeding lines of vegetable cowpea to find the association among characters. Results revealed that pod yield per plant showed strong positive correlation with number of peduncles and pods per plant, pod weight, pod length, number of seeds per pod and number of primary branches per plant, while negative correlation with days to 50% flowering at genotypic and phenotypic levels. The maximum direct positive effect on pod yield per plant was found contributed by number of pods per plant followed by pod weight.

No significant correlations were observed among traits related to seed yield and nutrient and protein



content in an investigation on one commercial variety and twenty-three cowpea local populations collected from Greece (Lazaridi et al. 2017). However, plant height was negatively correlated with days to first flower, flowering duration, days to first mature pod, Ca and Mg concentrations, while was positively correlated with height to first pod, pod length, number of seeds per plant and seed weight per plant. Days to first flower were positively correlated with flowering duration, days to first mature pod and Ca seed concentration with a negative correlation to height to first pod and pod length. The number of pods per plant was significantly correlated with number of seeds per plant and seed weight per plant. Number of seeds per plant was correlated with seed weight per plant and hundred seed weight. While, seed crude protein content was positively correlated with number of branches and days to first mature pod. Magashi et al. (2017) indicated a positive relationship between the number of days to 50% flowering and number of days to maturity, similar relationship exist between number of days to maturity and number of pod per plant and also between number of days to 50% flowering and number of pod per plant, pod length and number of days to 50% flowering, seed per pod and number of days to 50% flowering, seed per pod and number of days to maturity, pod per plant and pod length a positive relationship also exist. Similarly, in the result indicated that positive relationship exist between 100 seed weight and number of days to flowering, days to maturity, pod per plant, pod length and seed per pod. A positive relationship was also found between leaf area and seedling height, pod per plant and 100 seed weight. However negative relationship was between plant and other parameters.

An investigation of Srinivas et al. (2017) on 30 genotypes of cowpea were carried out during summer season in the year 2014-2015. The result on phenotypic and genotypic correlation coefficient revealed that pod yield per plot was significantly and positively correlated with number of branches per plant (0.7659), number of nodes (0.5523), pod length (0.3960), number of seeds per pod (0.2815), number of cluster per plant (0.550), number of pods per plant (0.547), number of pods per cluster (0.524), plant height (0.437) and protein content (0.2871). However, days for 50% flowering (-0.2081)showed significantly and negatively correlated with pod yield per plot. Other characters viz., days taken for first flowering (0.1946), pod diameter (-0.1035)showed negative non significantly correlated with pod yield per plot. Path coefficient analysis of different yield and yield contributing traits on number of branches per plant, number of nodes per plant, number of clusters per plant, number green pods per plant, number of pods per plant, number of seeds per pod, pod weight (g), pod yield per plot and percentage of protein content exhibited positive direct effects on pod yield per plot these characters play a major role in recombination breeding and suggested that direct selection based on these traits will be rewarded for crop improvement of cowpea.

In a study, Ngoc et al. (2019) reported the seed yield per plant highest and positive significant correlation with number of pods per plant, pod yield per plant, number of clusters per plant, number of branches per plant, number of pods per cluster, pod weight, number of seeds per pod and harvest index. If the selection is made any of the component traits, simultaneous selection of the all the traits could be achieved. The estimates of correlation coefficient revealed that the biological yield per plant was significantly and positively associated with plant height, days to 50% flowering, days to maturity, pod length, pod weight and number of seeds per pod. Among all the traits under study, harvest index, biological yield per plant, pod yield per plant, number of seeds per pod and number of pods per plant reflected high direct and positive effect of on seed yield per plant. This suggested that direct selection based on these traits would result in higher breeding efficiency for improving seed yield per plant. Thus, these traits might be estimated as the most important component traits for seed yield per plant. Number of seeds per pod had shown negative indirect effect through pod length but positive indirect effect via biological yield per plant. Plant height and biological yield per plant had high negative indirect effects via harvest index on seed yield per plant. Similarly, harvest index revealed this type effect on seed yield per plant via biological yield per plant.

Ngoc et al. (2019) had led to improve the understanding many interrelated processes involved the genetic control of variation and seed yield thus would provide some guidelines in selection, in the prediction of possible advantages of genetic recombination and for selection in segregating generations.

### Genetic divergence using D<sup>2</sup> analysis

Borah and Fazlullah Khan (2001) studied sixty genotypes of fodder cowpea (*Vigna unguiculata* (L.) Walp) for the genetic diversity. They were grouped into 10 clusters indicating high genetic divergence among cowpea genotypes. The clustering pattern showed that geographic diversity is not an index of genetic diversity. Intra cluster distance exhibited a range of 13.59 to 16.01. The maximum inter-cluster distance (D) was noticed between cluster I and X (41.60). It was least between cluster V and VI (17.52). Based on the intercluster distance and cluster mean for various characters, it could be seen that the cluster I, II, IX and X were the most divergent from the other clusters. The genotypes from these clusters may possibly be utilized for hybridization programme. Dry matter yield, green fodder yield and plant height contributed highly towards the genetic divergence among the types studied.

The D<sup>2</sup> analysis of 155 cowpea lines revealed that genotypes exhibited considerable diversity and were grouped in 10 clusters. Cluster I exhibited maximum number of germplasm lines. Inter-cluster distance was maximum between clusters IV and X followed by clusters IX and X. Cluster IX recorded highest mean seed yield per plant, 100-seed weight and also the intra-cluster distance. The genotypes belonging to these clusters viz., GC 3 and DCP 10 may be useful in multiple breeding programme to recover transgressive segregants with the desirable combinations of yield components (Saini et al. 2004). Cowpea 127 germplasm lines were grouped into 10 clusters on the basis of D<sup>2</sup> analysis. Cluster I exhibited maximum number of genotypes. Inter-cluster distance was maximum between clusters VIII and X followed by clusters IV and VIII. Intra-cluster distance was maximum in cluster VIII. The clustering was useful to identify the diverse genotypes. Cluster IX registered the highest mean value for plant height (88.74 cm), seed yield per plant (35.69 g) and 100-seed weight (14.93 g) (Jain et al. 2006).

Girish et al. (2006) evaluated 100 cowpea genotypes to quantify the genetic diversity existed among them. The genotypes fell into 11 clusters. Among the 11 quantitative characters studied, fodder yield contributed highest (75.73%) towards the divergence followed by plant height (8.28%) and seed yield (6.3%). Cluster VI had minimum days to first flower opening and days to maturity and also had maximum number of pods per plant, pod length, number of seeds per pod and seed yield. Cluster IX exhibited lowest means for seed yield, fodder yield, pod length, number of seeds per pod, primary branches and plant height. The genotypes from clusters VI and IX, which have high and low cluster means for majority of the characters are suggested as promising parents for hybridization. Bhandri and Verma (2007) carried out divergence analysis on 22 genotypes and grouped into seven clusters. The maximum intercluster distance found between cluster V and VII. Crude protein, dry matter digestibility, dry matter yield and number of leaves per plant were major contributors towards genetic divergence.

Dalsaniya et al. (2009) studied diversity among 60 genotypes of cowpea which were grouped in to 12 clusters revealing the presence of considerable diversity in the material. The clustering pattern of the varieties usually did not confirm to geographical



distribution. The intra cluster distances of 12 groups were ranged from 0.0 to 15.0. The maximum inter cluster distance (D) was noticed between cluster IX and X (D = 55.42) followed by that between cluster X and XII (D = 51.83), while the closest proximity was observed between cluster VII and XI (D = 9.99) followed by the cluster VIII and XII (D = 10.86). It was also noted that genotypes of cluster-X which had higher cluster mean values for yield and other desired characters like leaf area, ten pods weight, number of pods per plant and green pod yield per plant etc. could be directly tested in multilocation trials for their suitability or could be used as a donor parent in breeding programme. The characters like plant height, green pod yield per plant, protein content and leaf area were found to contribute much to the total genetic divergence in cowpea.

The study was done on cowpea genotypes with upright growth and early maturity using multivariate techniques to estimate the relative contribution of the response variables to genetic divergence studied. Twenty-eight lines of the Genbank of the Universidade Federal do Ceará and Embrapa Meio Norte were used. For the analysis of genetic divergence, the canonical variables, Mahalanobis' distance and the Tocher cluster method were used. Most of the maximum distances were observed when combined with CE-46 genotypes. The 28 genotypes were separated into 11 groups. There was a wide distribution of genotypes in different groups, indicating a wide diversity among the genotypes. The greatest distances were observed between the groups VIII and XI (343.2) and VII and VIII (323.2). The lowest D<sup>2</sup> distance values were observed in the intercross between the groups VII and XI (22.5) and VI and IX (36.4). The variables beginning of flowering and crop cycle contributed most to the genetic divergence among the genotypes (Dias et al. 2009).

Sixty-six genotypes of cowpea were investigated to understand the extent of genetic diversity through twelve quantitative traits. Mahalanobis's D<sup>2</sup> analysis established the presence of wide genetic diversity among these genotypes by the formation of 23 clusters. Cluster I had the maximum number of genotypes *i.e.* 22 and cluster 23 had only one genotype. The minimum intra cluster distance was observed in the cluster II. The inter-cluster distance (D) was found to be the maximum between the clusters XXII and XXIII and the same was minimum between clusters II and V. The results indicated that grain yield per plant contributed maximum to the total divergence followed by 100 seed weight and days to 50% flowering. Number of branches per plant had least contribution to the total divergence followed by petiole length. The existence of wide genetic diversity among the types chosen

from the same geographical location was obviously seen. In this study, the variety Vellayani local had the maximum value for plant height and pod length and thereby distinguished from other varieties and it is present singly in the cluster XXIII. The clustering pattern of the varieties in the present study clearly indicated that there was no parallelism between genetic and geographic diversities (Nagalakshmi et al. 2010). According to (Costa et al. 2013), the dissimilarity measures were ranged from highest to shortest in the line pairs IT82D-889 and IT89KD-245 (221.35) and IT98K-128-4 and IT97K-1042-3 (1.21), respectively. Based on the UPGMA analysis, the lines were distributed into four distinct groups, which consisted of few lines, with the exception of the fourth group that contained 71% of the genotypes studied.

Nancee et al. (2013) carried out experiment at Research Farm of the Department of Vegetable Science, CCS Haryana Agriculture University, Hisar during spring/summer season of the year 2011. Based on D<sup>2</sup> values, forty-six genotypes were grouped into eight clusters containing two to fifteen genotypes. These clusters consisted of genotypes with different geographical origins and indicated no correlation between genetic and geographical divergence. The genotypes of cluster VIII showed maximum genetic divergence with cluster V followed by Cluster II, hence, the genotypes belonging to cluster VIII and cluster V may be selected for generating genetic variability and hybridization. Cluster II having two genotypes was found to be the best performing for agronomic characters followed by cluster I with three genotypes and cluster V with three genotypes. Thus, to generate desirable genetic variability, the crossing between cluster II, I and V genotypes would be useful. The number of seeds per pod contributed highest towards divergence followed by plant height at final harvest (cm).

The present study, multivariate analysis was carried out by Ahamed et al. (2014) to assess the genetic diversity among eleven cowpea germplasm. Mahalanobis generalized distance (D<sup>2</sup>) analysis was used to group the cowpea geno-types. Considering the mean values, the germplasm was grouped into four clusters. Maximum numbers (4) genotypes were included in cluster III and a minimum number (2) genotypes were included in both cluster II and IV. Among the clusters, the highest inter-cluster distance was obtained between the cluster IV and II (23.952) and the lowest one was obtained between II and I (6.753). The maximum value of inter-cluster distance indicated that genotypes belonging to cluster IV were far diverged from those of cluster II. The first female

flower initiation was earlier in BD-8344 (97 days) than the other germplasm. BD-8348 produced maximum number of pods per plant (41.20). The highest grain yield per plant (66.65g) was recorded from entry BD-8344 and the lowest grain yield per plant (28.80g) was also obtained in BD-1604.

Brahmaiah et al. (2014) evaluated forty cowpea genotypes for 18 quantitative characters to estimate the genetic diversity existing among them by using Mahalanobis D<sup>2</sup> statistics. The genotypes were grouped into six clusters. The cluster strength varied from single genotype (Clusters III, IV and V) to 25 genotypes (Cluster I). Clusters IV and VI had high inter cluster distance. Clusters II, III and I had maximum 100-seed weight, number of seeds per pod and seed yield respectively. Cluster IV had maximum seedling vigour index, germination per cent, peduncle length, number of clusters per plant and number of primary branches. The genotypes from clusters IV and IV may be inter crossed to obtain high variation. Chattopadhyay et al. (2014), grouped into cowpea genotypes in seven clusters, cluster I had maximum of 7 genotypes, Cluster II, III, IV and V comprised of 2 genotypes each, while Cluster VI and VII had one genotype each. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection are responsible for genetic diversity. The maximum inter-cluster value was observed between cluster I and VI (85.245) followed by 84.974 between Cluster III and VI which indicated that the genotypes included in these clusters had the maximum divergence. Cluster II had genotypes with high pod productivity, while the genotypes in Cluster III flowered much earlier. Crossing between genotypes within these groups could produce highly productive and early maturing cowpea genotypes.

The investigation on genetic diversity studies for seed yield in cowpea was conducted by using 44 genotypes of cowpea. Nath and Tajane (2014) have reported that there was substantial genetic diversity among the genotypes studied. 44 genotypes were grouped into 6 clusters to study the genetic divergence for seed yield per plant. There was no parallelism between genetic diversity and geographical distribution. For seed yield, the pair of genotypes *viz*., Phule CP 05001 and UPC-5286 were most divergent from one another (D<sup>2</sup>=1225.35). On the basis of inter-cluster distance, cluster means and per se performance observed in the present studies, following genotypes are suggested for hybridization to improve seed yield in cowpea. 1.UPC-5286, 2.Phule Pandhari, 3.NBPGR- 05-66, 4. Pusa-do-fasali, 5.Shweta, 6.NBPGR-05-67, 7.CP-23-GPM, 8.NBPGR 05-71.

Sandeep et al. (2014) carried out genetic divergence using D<sup>2</sup> analysis in 50 diverse genotypes of cowpea. All the 50 genotypes were grouped into twelve clusters. Cluster I was largest comprising of twenty seven genotypes followed by Cluster II with twelve genotypes, cluster IV with three genotypes and cluster III, V, VI, VII and VIII, IX, X, XI, XII were represented each by single genotype. Intra-cluster D<sup>2</sup> values ranged from 0 to 38.06. The inter-cluster D<sup>2</sup> values ranged from 44.08 to 276.55. The maximum inter cluster distance was observed between VII and XII clusters followed by clusters IV and XII and cluster X and VII. The maximum contribution towards genetic divergence is by days to 50% flowering (25.22%) followed by plant height (12.24%) and biological yield per plant.

Forty-four genotypes of yard long bean (Vigna unguiculata subsp. sesquipedalis (L.) Verd.) were investigated to understand the extent of genetic diversity through twelve quantitative traits. Mahalanobis's D<sup>2</sup> analysis established the presence of wide genetic diversity among these genotypes by the formation of 3 clusters. Cluster I had the maximum number of genotypes *i.e.*34 and cluster III had only four genotypes. The inter-cluster distance (D) was found to be the maximum between the clusters II and III and the same was minimum between clusters I and II. The results indicated that 100 seed weight contributed maximum to the total divergence followed by pod yield per plant. Intercrossing among the genotypes belonging to cluster II, V and IV was suggested to develop high yielding varieties with other desirable characters or may be used as potential donors for future hybridization programme to develop superior yard long bean variety with good consumer preference and high pod yield (Vavilapalli et al. 2014a).

In another study, Vavilapalli et al. (2014b) in order to assess the divergence among 22 cowpea genotypes, Mahalanobis  $D^2$  statistics was applied. The 22 genotypes were grouped into 6 clusters, where clusters I was the largest, containing eleven genotypes followed by the clusters III (5 genotypes) and cluster II with three genotypes. The inter cluster distance was maximum between cluster III and VI followed by cluster III and V. Based on inter cluster distance and per se performance of genotypes, the entries viz., VU 1, VU 2, VU 6, VU 8 and VU 21 were selected, which could be intercrossed to recover good recombinants and desirable segregants. The pod yield per plant contributed maximum divergence (66.23%) which was followed by pod weight (20.78%) and plant height (8.23%). In another study, ten cultivars were



divided into two broad genetic groups (A&B) based on squared Euclidean distances. Group A consist of two clusters which members showed low performance in terms of economic traits and group B comprised two clusters of superior cultivars for most of the traits studied (Animasaun et al. 2015).

Results of Chandrakar et al. (2016) on twenty-one characters showed low quantum of divergence among 21 genotypes of vegetable cowpea. These cowpea genotypes were grouped into five clusters on the basis of D<sup>2</sup> analysis. Maximum number of genotypes (9) was accommodated in cluster-II. The average inter and intra cluster divergence (D) values had also been calculated. The maximum inter cluster distance was observed in between cluster I and V (5.04). The cluster III showed maximum value for mean green pod yield (146.820g.) followed by cluster IV (146.156g). Crossing between the genotypes of maximum two clusters appeared to be most promising to combine the desirable characters. Srinivas et al. (2016) grouped 30 genotypes of cowpea into six clusters. Maximum inter cluster D<sup>2</sup> value was observed between VI (6987.85) and III (4806.87), indicating that the genotypes included in these clusters had maximum divergence. The diversity among the genotypes measured by inter-cluster distance was adequate for improvement of cowpea by hybridization and selection. Asoontha and Mareen, (2017) grouped 12 genotypes of yard long bean into five clusters. The inter cluster distance was maximum for cluster I and cluster II (10.93) followed by cluster II and cluster V (8.91) and cluster II and cluster III (8.87). The least intercluster distance was recorded between cluster I and cluster IV. The intracluster distance was highest for cluster III followed by cluster II and cluster I. The intracluster distances was least and zero for cluster IV and cluster V as they are the solitary ones.

Study on 66 advance breeding lines of vegetable cowpea, Lal et al. (2017) proposed divergent occurrence among these genotypes. Based on degree of divergence the genotypes get grouped into seventeen clusters. The top three characters which contributed most towards the genetic divergence were number of peduncles per plant, peduncle length and pod length. The genotypes of cluster VII showed maximum genetic divergence with genotypes of cluster VIII. Further, cluster VII had the high yielding genotypes, while cluster XVII had early flowering genotypes. (Lovely et al. 2017) conducted a study on nature and magnitude of genetic divergence among 50 genotypes of vegetable cowpea collected from different agro climatic regions of South India. All the genotypes were grouped into four clusters with genotypes from different geographic locations being grouped in the same clusters. The cluster I had

the highest number of genotypes. The genotype VS41 remained in a solitary cluster as a divergent genotype that cannot be accommodated in any of the clusters. Pod yield per plant contributed the maximum towards divergence. Patel et al. (2017) evaluated 32 cowpea genotypes by using D<sup>2</sup> statistics. The genotypes were grouped into eight clusters. The maximum intercluster distance (D<sup>2</sup>=35.43) was observed between cluster-VI and VIII. Clusters II, III and I had maximum 100-seed weight, number of seeds per pod and seed yield respectively. Therefore, it was concluded that the genotypes belonging to these cluster should be intercrossed in order to generate more variability. Vu et al. (2017) studied cluster pattern in cowpea and revealed that highest inter-cluster average D<sup>2</sup> values among genotypes existed between clusters III and VI (52.08). Therefore, the crossing between the genotypes of most divergence clusters i.e. III (KBC-8, GC 901, VCP 09-019, KBC-6, PTB-1, UCP 12-007) and VI (TC 150) could able to produce high seed yielding transgressive segregants. It would be logical to attempt crosses between the diverse genotypes belonging to clusters separated by large inter-cluster average D<sup>2</sup> values. This clearly showed presence of wide variation from one cluster to another in respect of cluster mean for six clusters.

In a meterolygraph study of 30 cowpea genotypes, Arya et al. (2019) placed KBC 9 and DC 7-15 in the medium fodder-medium grain yield producing group and could be utilized for both, fodder as well as grain production. Moreover, the genotypes (UPC 12-007, KBC-6, KBC-8 and GC 901) had good potential of grain production as well as excellent potential of fodder production. Likewise, the genotypes (PL-3 Sel., CPD 240 and RC 101) were included in medium fodderhigh grain yield, which had good potential of grain production as well as excellent potential of fodder production. The hybridization among the diverse genotypes viz., UCP 12-007 (high fodder yield) with PL-3 Sel. (high grain yield) may be suggested for developing dual purpose genotypes through the transgressive segregation.

### Genetic diversity in cowpea by using molecular markers

Assessment of genetic variability within cowpea is fundamental for the conservation of genetic resources and its utilization in hybridization programme. The use of new tools of molecular biology like diversity studies can overcome some of the breeding program limitations and speed up selection time of new varieties for crosses. Different molecular techniques were used for diversity study of wild and cultivated cowpea which included Amplified Fragment Length Polymorphisms (AFLP); Chloroplast DNA Polymorphisms; Random Amplified Polymorphic DNA (RAPD) Restriction Fragment Length Polymorphisms (RFLP); DNA Amplification Fingerprinting (DAF); and analysis of Simple Sequence Repeats (SSRs) or Sequence Tagged Microsatellite Sites. Of these techniques, analysis of SSRs has proven to be particularly useful since these sequences are abundant and distributed throughout eukaryotic genomes (Doumbia et al. 2014).

In a study, Ba et al. (2004) used RAPD analysis to characterize genetic variation in domesticated cowpea and its wild progenitor, as well as their relationships from West, East and southern Africa. A total of 28 primers generated 202 RAPD bands. 108 bands were polymorphic among the domesticated compared to 181 among wild/weedy cowpea accessions. Wild accessions were more diverse in East Africa, which is the likely area of origin of V. unguiculata var. spontanea. var. spontanea is supposed to have spread westward and southward, with a loss of variability, loss counterbalanced in southern Africa by introgressions with local perennial subspecies. Although the variability of domesticated cowpea was the highest ever recorded, cultivar-groups were poorly resolved and several results obtained with isozyme data were not confirmed here. However primitive cultivars were more diverse than evolved cultivars, which still suggest two consecutive bottlenecks within domesticated cowpea evolution. As isozymes and AFLP markers, although with a larger number of markers, RAPD data confirmed the single domestication hypothesis, the gap between wild and domesticated cowpea and the widespread introgression phenomena between wild and domesticated cowpea.

Genetic relationships among elite lines of cowpea were assessed using four enzyme systems and 10 random oligonucleotide primers. Multimeric isozyme profile and polymorphic RAPD markers reflected presence of considerable amount of genetic variability among the genotypes. Keeping in view the need for developing varieties with early maturity, determinate growth habit and high yield and root rot disease resistant and susceptible lines were used in the present investigation to assess their genetic relationships. Except superoxide dismutase (SOD), other three-isozyme systems, peroxidase (PRX), esterase (ESt) and polyphenol oxidase (PPO) yielded polymorphic bands. The cluster analysis showed that genotypes could be grouped into three main clusters. The level of polymorphism observed with both RAPD and isozyme indicated diversity in genotypes for root rot resistance. RAPD analysis indicated that amongst the released varieties, Bundel lobia-1 was most distinct. Its similarity coefficients with Bundel

lobia-2, Kohinoor and UPC-5286 were 79, 78 and 75 per cent, respectively (Sahay et al. 2008).

RAPD markers were used to assess the genetic diversity among selected genotypes of Vigna unguiculata. Out of 35 RAPD primers tested, 14 allowed amplifications of random polymorphic (RAPD) loci. A total of 113 amplified products were obtained out of which 16 were monomorphic and 97 were polymorphic. Average polymorphism across 24 genotypes was found to be 79.87 per cent. For the genotypes tested, 2 to 14 bands were obtained, with an average of 8.007 bands per primer. The 24 genotypes were grouped into two major clusters at a similarity coefficient of 0.50. Genetic similarity matrices of the genotypes ranged from 0.3462 to 0.8681, indicating a high genetic variability among the genotypes. Similarity value for all other genotypes varied between the two extreme values. The results indicated that RAPD markers were efficient for the identification of genotypes and for determination of genetic relationships among them (Malhotra et al. 2009). Patil et al. (2013) estimated the genetic diversity of thirty genotypes of cowpea in a study by using RAPD markers. RAPD profiles for 30 genotypes were generated with 20 random decamer primers. Out of 20 primers screened 17 primers gave scorable DNA fragments and each of the 17 primers revealed various levels of polymorphism. These

primers generated 1238 DNA fragments in the average range of 381.94 bp to 1131.71 bp, of which 908 were polymorphic. The level of polymorphism among the genotypes was found to be very high (71.20%). The overall range of similarity among 30 genotypes was found to be very wide, ranging from 0.321 to 0.800 which indicates there was high variability among the cowpea cultivars under study.

The genetic diversity of cowpea in Ethiopia was analyzed using 19 uniform accessions, 62 variable accessions (yielding 185 sub-types) and two mung bean accessions (four subtypes) as out-group. A set of 23 polymorphic SSR markers was identified and polymorphism was scored. A total of 75 allelic variants was defined, with the average number of alleles per locus calculated to be three. The average genetic diversity (D) was 0.47 and PIC was 0.4. The accessions showed no clustering by geographical origins. Three well-characterized molecular markers (SSR1, C42-2B and 61RM2) for race specific resistance to Striga gesnerioides in the cowpea cultivar B301 were used to evaluate the accessions for their potential for use in genetic improvement against this pest. Only two accessions, 222890-2 from Gambela and 286-2 from the Southern Nations, Nationalities and Peoples (SNNP) region, were found to cluster with B301 and contain the SSR1 resistance allele (Desalegne et al. 2016).

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### Floral and Molecular Characterization of Gamma Rays Induced Mutants in Oil-Bearing Rose (*Rosa damascena* Mill.)

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### ABSTRACT

This research was aimed to create the genetic variations for the selection of oil-bearing rose genotypes with the desirable floral features. For this reason, gamma rays at doses of 0, 100 and 200 Gy of radioactive Cobalt-60 were applied to oil-bearing rose seeds to create genetic variations. Finally, the floral and molecular characterization of oil-bearing rose plants derived from non-irradiated and irradiated seeds were carried out. A total of 48 genotypes including 17 from control (0 Gy), 18 from M100 (100 Gy) and 12 from M200 (200 Gy) together with the parental species *R. damascena* were used as genetic materials. Genetic analysis was performed by using 20 SSR primer pairs. After the electrophoresis, the bands were displayed in TIF format with the aid of the Biolab UV Tech gel imaging system. The genotypes and mutants grown from irradiated and non-irradiated seeds were significantly different with flower colours from white to dark pink and petal numbers from 5 to 100. These results also showed that the seeds in the open-pollinated flowers of oil-bearing rose had mostly heterozygous allele genes governing the floral traits. The efficacy of the SSR primers used to identify mutations was different. RA003a and RA034a primers were found to be more effective in mutation screening in oil-bearing rose genome. It was understood that the major effects of the gamma-ray irradiation would be a huge selection source to breed novel varieties.

Keywords: Rosa damascena Mill., mutation breeding, gamma-ray, SSRs.

### Introduction

Turkey is an important differentiation and gene center for *Rosa* genus (Yıldırım 2016). Oil-bearing rose or Damask rose (*Rosa damascena* Mill.) with about 30 pink petals is one of the most strongly scented rose species with characteristic floral scent molecules such as citronellol, geraniol, nerol and phenylethyl alcohol. Its main industrial products used in the perfume and cosmetics are rose oil, rose water, rose concrete and rose absolute, which are produced by hydrodistillation and solvent extraction processes (Anac 1984; Başer 1992; Bayrak and Akgül 1994; Aydınlı and Tutaş 2003; Aycı et al. 2005). Turkey, Bulgaria and Iran are the leading countries which meet more than 90 percent of the world oil-bearing rose production. Oil-bearing rose as an agro-industrial product has been cultivated in the Lakes Region of Turkey (Isparta, Burdur, Denizli and Afyonkarahisar provinces) since the last quarter of the 19<sup>th</sup> century. The production area of oil-bearing rose was about 3845 hectares and annual fresh flower production was 16,560 tons in the Lakes Region of Turkey in 2019 (TUIK, 2020).

*Rosa damascena* Mill. is an allotetraploid species (2n=4x=28) as a hybrid of *R. gallica* L. and *R. phoenicia* Boiss. (Gudin 2000). *R. damascena* 

was considered as allied to *R. alba, R. phoenicia* and *R. moschata* according to DNA analyses (Yıldırım 2016). The studies on the origin and genetics of *R. damascena* have focused especially on molecular DNA markers in recent years. Because classical genetic analyses based on phenological and morphological characteristics give very limited and inadequate information, therefore, it is absolutely necessary to be supported by molecular and biochemical techniques (Torres et al. 1993; Debener and Mattiesch 1999). For example, microsatellite genotyping demonstrated that *R. damascena* Mill. accessions from Bulgaria, Iran, India and old European Damask rose varieties possess identical microsatellite profiles, suggesting a common origin (Rusanov et al. 2005).

According to the studies on molecular analyses with RAPD, AFLP and SSR markers, while polymorphism indicating that genetic variation is not present among the individuals and populations of *Rosa damascena* plants under cultivation in Turkey and Bulgaria (Ağaoglu et al. 2000; Göktürk Baydar et al. 2004; Rusonov et al. 2005), a wide genetic diversity was determined by molecular markers among the *R. damascena* plants collected from Iran and its neighbouring areas including Syria and Pakistan (Pirseyedi et al. 2005; Babaei et al. 2007; Kiani et al. 2010; Alsemaan et al. 2011; Farooq et al. 2013).

Since oil-bearing roses have always been propagated vegetatively for hundreds of years, the current roses in the rose valleys of Turkey and Bulgaria are most probably clonal progenitors of the first planted oil-bearing roses which have been maintaining their primitive features to date. For this reason, superior or outstanding types of oil-bearing rose have not been selected through breeding in Turkey due to the lack of genetic variation among the plants under culture (Baydar et al. 2016). On the other hand, the phenotypic homogeneity caused by continuous vegetative reproduction makes it possible to produce rose oil with international standards (ISO 9842:2003) (Rusanov et al. 2009).

There is a need for different methods such as hybridization (inter- and intraspecific crosses) and induced mutation (physical or chemical mutagens) to create genetic variation in rose species including oil-bearing roses (Raev 1984; Tsvetkov 1984; Baydar et al. 2013). The other quite practical and effective methods causing genetic variation in oil-bearing roses are to derive progenies from the seeds of the plants grown only by vegetative propagation with their cuttings (Gudin 2003). Many of the seedlings derived from the seeds of open-pollinated flowers may differ genetically due to the segregation of the alleles



at heterozygous loci during meiosis (Rusanov et al. 2005; Baydar et al. 2016). Microsatellites or simple sequence repeats (SSRs) are simple sequence of tandemly repeats which can presently be a short motif of di-nucleotides, or tri-nucleotides, or tetranucleotides repeated and contains in 2-6 base pairs (bp) in length (Li et al. 2004). Simple sequence repeats of few base pairs in length can find polymorphism of DNA from mutant populations. The type of DNA polymorphism could be detected only after polymerase chain reaction (PCR), amplification of DNA and separation on polyacrylamide gel electrophoresis (Wu and Steven 1993). Size polymorphism reflects variation in the number of repeats of a simple DNA sequence ranging in length from 2-6 base pairs (Chapuis and Estoup 2006). SSRs have become a popular type of codominant molecular marker in genetic analysis and plant breeding application and this marker system is very useful for the determination of variation among plant populations.

The primary objective of this study was to create, evaluated and molecular characterization of the genetic variations for the selection of the oilbearing rose genotypes with the desirable floral features. Therefore, gamma rays at doses of 0, 100 and 200 Gy of radioactive Cobalt-60 were applied to the oil-bearing rose seeds to create genetic variation. The floral traits such as petal color and petal number were evaluated and molecular characterization in the oil-bearing rose plants, derived from non-irradiated and irradiated seeds with <sup>60</sup>Co gamma-rays was performed by using simple sequence repeats (SSRs) to find polymorphism of DNA from mutant populations.

### Materials and Methods Plant materials

This research was conducted at the Faculty of Agriculture, Isparta University of Applied Sciences in Isparta-a city in the south-western part of Turkey which is called "Rose Valley of Turkey" due to the presence of advanced industrial oil-bearing rose cultivation. The seeds were extracted from the mature fruits of Rosa damascena Mill. f. trigintipetala Dieck (2n=4x=28) in Isparta region in October 2007. Following the irradiation of the seeds at different doses (0, 100 and 200 Gy) of Cobalt-60 gamma-ray in Nuclear Agricultural Research Center of Ankara, Turkey, they were sown to viols in March 2008. Healthy seedlings were transferred to pots first, then grown under greenhouse conditions in 2009 and eventually planted with 1.5 m within rows and 3 m between rows with drip irrigation applied to the experimental field in March 2010 (Baydar et al. 2016).

In subsequent years (2012, 2013 and 2014), basic floral traits as petal color and petal number per flower were determined on recently opened flowers in the early morning hours during the flowering season (May and June). Five flowers from each plant were measured on the condition that the plant produced enough flowers. Petal color was assessed on recently opened flowers and measured with a portable colorimeter (Model CR-300, Minolta Camera Ltd., Osaka, Japan) as described by Sarı (2018). Color was measured in the middle of each petal (three replicates per flower) to ensure equal measurement conditions (Schmitzer et al. 2010). The fragrance density and scent molecules (not tabulated in this paper) of the fresh flowers were detected using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography/mass spectrometry (GC-MS) with the help of the method explained by (Baydar et al. 2016).

The genetic analyses were also performed in total of 48 genotypes including 17 from control (0 Gy), 18 from M100 (100 Gy) and 12 from M200 (200 Gy), together with the parental species *R. damascena* Mill, by the use of SSR markers. The band patterns of each selected mutant representing each application dose of Co-60 were compared to the band pattern of control and parental plants.

#### **DNA** extraction

DNA was extracted from the fresh leaves and shoots of the plants using a Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions. The DNA quantities  $(ng/\mu L)$  were determined spectrophotometrically using the Thermo Scientific NanoDrop<sup>TM</sup> Spectrophotometer. The DNA quality was measured by the ratio of the absorbance values of 260 nm and 280 nm. The extracted DNA was stored at  $-80^{\circ}$ C until use.

### SSR analysis

Simple Sequence Repeat (SSRs) primers which were previously designed for Rosa species by Hibrand-Saint Oyant et al. (2008) and Kimura et al. (2006) and also used in this study were shown in Table 1. PCR amplification protocol used in this study was previously successfully applied in *R. damascena* by Göktürk Baydar et al. (2004). Each PCR reaction was prepared as follows: 25 ng template DNA, 5 pmol of each labelled reverse primer and unlabelled forward primer, 1U Tag DNA polymerase, 2 µL of 10×reaction buffer, 1  $\mu$ L of 1×W1-detergent, 2  $\mu$ L of dNTPs (1 mM), 2 µL of magnesium chloride (15 mM) in a total volume of 20 µL. The PCR reactions were carried out in a BioRad thermocycler. An initial denaturation of 94°C/30 s was followed by 30 cycles. Denaturation and extension temperatures were 94°C for 1 min

and 72°C for 2 min, respectively. The annealing temperature (about 58°C for 50 s) changed according to primer pairs used. The amplified fragments were separated in the electrophoresis by 6% polyacrylamide gel using 29:1 acrylamide:bisacrylamide solution and 5% tris boric acid EDTA buffer and stained with ethidium bromide. The bands were displayed in TIF format with the aid of the Biolab UV Tech gel imaging system.

### Results

Floral characteristics, DNA quantity and quality and PCR amplification of 20 SSR primers tested in the *Rosa damascena* genotypes were shown in Table 2. The genotypes which were derived by generative propagation with seeds and were gamma-irradiated with Cobalt-60 had a huge floral variation for the flower colors from white to dark pink and the petal numbers from five to a hundred as shown in Figure 1. While the petal color of the classical Isparta oilbearing rose was pink, the petal color of the genotypes used in the study changed from white to different shades of pink. White petal color occurred in the Rd-M0 group (52 code), Rd-M100 group (18, 28/1 and 100 codes) and also Rd-M200 group (34 code) (Table 2).

It was observed that genotypes with more petals had fewer anthers (the pollen producing part of a flower) and genotypes with more anthers had fewer stigmas (the part of the pistil where pollen germinates). In general, those with a low number of petals (single-layered flowers with 5-7 petals) tend to bloom earlier than those with a higher number of petals (multi-layered flowers with over 25 petals). It was also observed that the flowering season of those with a low number of petals was shorter. This finding was important to show that there were close relationships between the number of petals and flowering duration according to the correlation analysis results (not tabulated). On the other hand, the flowers of single-layered genotypes were very light and had lower odor densities as well as their petal leaves were not suitable for hand collection because they were swallowed very quickly (Baydar et al. 2016). Another remarkable point by visual and sensorial inspections was that the genotypes with hairless (naked) hypanthia, pedicels and sepal leaves were less fragrant but more resistant to rose aphid (Macrosiphum rosae L.).

DNA quantity varied from 6.40 to 95.4 ng/ $\mu$ L and DNA quality (260/280 values) changed from 1.61 to 2.40 (Table 2). A total of 1920 (48 genotypes x 20 primers x 2 dose mutations) PCR reactions

were performed for the SSR analysis and a total of 100 polyacrylamide gels were prepared for the screening of the amplification products. Different SSR primers detected different locus losses resulted from chromosomal deletions (Table 3). All SSR markers used in the study were amplified in the parental genome of Rosa damascena. Among the SSR primers, RA003a (primer 8) and RA034a (primer 16) were found to be the most effective primers in oil-bearing rose genome mutation screening (Table 3). When the reaction products obtained were examined, it was determined that SSR primers being specific to locus amplified a single allele in all of the genotypes examined in the study (Figure 2a). Polymorphisms mostly occurred in the form of deletion of the entire SSR locus when compared to R. damascena (control) genome (Figure 2b). However single mutant also exhibited shortened specific SSR locus region due to the break on its genetic material (Figure 2c). Rd-M100-29 was identified as a mutant with the highest number of chromosomal deletions including 5 different locus losses (Table 3).

### Discussion

The irradiation of gamma rays of Cobalt-60 to oil-bearing rose (Rosa damascena Mill.) seeds produced a broad genetic variation in the progenies in terms of floral traits like petal color and petal number (Figure 1, Table 2). These variations can be a very important genetic source for rose breeding to develop novel oil-bearing rose varieties. Even in the plants from the non-irradiated seeds (coded as M0), there is a wide variation in these traits. A wide variation in floral scent molecules such as phenyl ethyl alcohol (23.26-74.54%), citronellol (5.57-31.59%) and geraniol (3.09-26.93%) apart from the floral morphological characteristics was recorded among the seed-derived (non-irradiated) oil-bearing rose plants (Baydar et al. 2016). R. damascena Mill. is mainly accepted as a hybrid of *R. gallica* L. and *R. phoenicia* Boiss. (Gudin 2000). It is also thought to be a triparental origin of R. gallica, R. moschata and R. fedtschenkoana (Iwata et al. 2000), both of which support our observations. Our results demonstrated that oil-bearing rose may have originated from more than three parental species during the evolution for thousands of years. However, much more detailed molecular genetic studies are needed to prove this hypothesis.

The petal color was changed from white to dark pink (Table 2). However, the majority of the genotypes and the mutants had pink petal color like the parent *Rosa damascena* grown in the experimental field. This finding supports the idea that the pink color is a



dominant character over other petal colors. The pink color has been shown to be inherited codominantly, with white being homozygous recessive, pink being heterozygous and darker pink being homozygous dominant, as previously confirmed by Jones (2013). The coloration of rose flowers is mainly caused by the accumulation of anthocyanins such as pelargonidin and cyanidin in the petal cells (Schmitzer et al. 2010). Roses lack blue/violet flower colors owing to the deficiency of F3'5'H and therefore lack the B-ring-trihydroxylated anthocyanins based upon delphinidin (Tanaka et al. 2008). Karami et al. (2012) found a high positive correlation (r Sq Linear=0.81) between essential oil content and anthocyanin concentration which can be used as an essential oil quantity index in R. damascena. It has also been reported by Nedkov et al. (2009) that the essential oil content of white rose (Rosa alba) is lower than that of pink rose (Rosa damascena).

The petal number per plant was varied from 5 to 100 (Table 2). The important and negative relationship between the number of petals and the number of anthers may be the result of the homeotic functions of MADS-box function genes such as A, B and C. As known in this model, the formation of the floral organs is controlled by three sets of functional genes from the MADS-box gene family as A (sepal and petal formation), B (petal and stamen formation) and C (stamen and carpel formation) that are expressed in certain regions of the developing flower (Causier et al. 2010). While wild roses have simple flowers typically with 5 petals per flower, modern roses have double flowers consisting of >10 petals (Bendahmane et al. 2013). The roses with double flowers might be due to a homeotic change of stamens into petals through the concept of a sliding boundary, which is also responsible for the morphological diversity of rose flowers (Dubois et al. 2010).

Because the oil-bearing rose is an allotetraploid (2n=4x=28) carrying 4 homolog chromosomes that are homologs of each other, the same gene locus is replicated at the tetrasomic level and the genome of which is highly heterozygous. As a result, the genetic variations resulting from the segregation of the alleles at heterozygous loci were also appropriate for the clonal selection of novel varieties. However, it is not easy to recognize the genetic variations caused by heterozygous alleles or gene mutations. Several different types of DNA markers may be used to determine the genetic differences in the variants obtained after mutation (Chakrabarty and Datta 2010).

In this study, twenty SSR primer pairs were used to screen the genetic structure in a population of 47 oil-bearing rose genotypes together with *Rosa*  *damascena* Mill.. It has been understood that SSR analysis is an effective and practical method to identify the genetic variation in oil-bearing mutant populations. There are also some researches support on the SSRs are effective primers in screening for mutations in genomes. For example, mutations caused by somaclonal variations in rice plants were successfully detected with SSR primers (Khai and Lang 2005).

However, the efficacy of the primers used to identify mutations was different in oil-bearing rose genomes (Figure 2, Table 3). RA003a and RA034a primers were found to be the most effective markers in mutation screening among other microsatellite markers. Microsatellites can be found in thousands of locations in a plant genome; in addition, it has a higher rate of mutation than other DNA regions leading to high genetic diversity. However, microsatellites cannot be amplified by PCR due to the point mutations or false base pairings where SSR primers hybridize and the expected polymorphisms may not be evident if false alleles (null alleles) cannot be distinguished. Moreover, highly polymorphic microsatellite markers are widely employed in population genetic analyses, but one potential drawback is the presence of null alleles that fail to amplify to detected levels in the PCR (Dakin and Avise 2004).

In this research, the Damask rose genotypes and mutants with distinctly different in petal color and number were distinguishable by SSR markers. However, small floral differences caused by point mutations are not always detected by SSR markers which only allow scanning a very small fraction of the rose genome (Göktürk Baydar et al. 2004). Sport mutants with clearly mutated phenotypes show identical DNA marker patterns as the single mutations leading to the altered phenotypes are derived from only a negligible small part of the DNA of the mutant genotype (Debener et al. 2000). Unlike point mutations, which affect only a single nucleotide, microsatellite mutations lead to the gain or loss of an entire repeat unit and sometimes two or more repeats simultaneously. However, since the genome analysis and the genetic map of Rosa damascena have not yet been prepared, SSR markers developed for other rose species such as Rosa hybrida were used successfully. In addition, with Single Nucleotide Polymorphism (SNP) markers developed by direct sequencing of PCR products obtained from the genomic DNA of Rosa damascena, more detailed information on the allele configurations of genes related to floral properties can be obtained (Rusanov et al. 2009). Furthermore, TILLING (Targeting Induced Local Lesions IN Genomes) is a well-known reverse genetics technique designed to detect unknown SNPs in genes of interest using enzymatic digestion and is widely employed in plant genomics (Wang and Shi 2015).

The results from this study showed a huge floral variability for both petal color and number among the oil-bearing rose genotypes derived by generative propagation with seeds and induced gamma-irradiation with 60Co. Open-pollinating flowers of the oil-bearing rose show a high degree of outcrossing and therefore lead to a high degree of heterozygosity in the alleles or genes associated with important floral characteristics and scent molecules. So, the seed propagation of clonally-propagated R. damascena yields a change in the genetic segregation of the alleles especially related to the petal color and petal number. Consequently, SSR markers linked to floral characteristics of oilbearing rose should be developed from the segregating population of oil-bearing rose and also more effective methods such as SNP and TILLING are needed for the high-throughput identification of the mutants with a range of modified functions for a particular floral gene. Instead of screening all of the base sequences of an entire R. damascena genome, it has been concluded that it could be better to determine the base sequences only in the related floral genes for genome comparisons.

### Conclusion

In oil-bearing rose industry, since the rose flowers constitute the main parts of the production costs in the basic distillation and extraction products such as rose oil, rose water, concrete and absolute, it is important to develop novel oil-bearing rose varieties with high flower yield and high volatile oil yield for reducing the production costs. Due to the lack of sufficient genetic variations among the oil-bearing roses under culture, until now it was not possible to select superior or outstanding types by certain breeding programs in Turkey. This research reveals that seed propagation and mutation breeding can be easily utilized as a way to create effective genetic variations for floral and scent characteristics. In conclusion, oil-bearing rose seeds with or without gamma-ray irradiation could provide a huge selection source to breed novel varieties.

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Figure 1. Floral images of some *Rosa damascena* genotypes derived from non-irradiated and irradiated seeds with <sup>60</sup>Co gamma rays. In the figure, Rd refers to *Rosa damascena*; M refers to mutation; 0 (control), 100 and 200 refer to gamma ray doses. (Original)



Figure 2. a) The gel image of PCR products of SSR primer Rw5G14 in polyacrylamide gel; b) Loss of locus by Rw12J12 primer in Rd-M100-15; c) a shortened locus area by RA013a primer in Rd-M200-28.





No	Primers	SSR Motifs	Sequence of Primers
1	Rw5G14	(CT) <sub>7</sub> (C) <sub>8</sub>	F: TGGTTTGGGGGTTTTGTGTCT R: GCACAGTCTCCACCTGACAA
2	Rw12J12	(CT) <sub>11</sub>	F: CAGTGTCCATGCTGACGAGT R: TGCTCCTGTTTTCTCTTTGCT
3	Rw55E12	(TC) <sub>9</sub>	F: CGGTGGTTGGACATTAAAGC R: GGAGGCAACAGCACACTCTC
4	Rw16E19	(TTC) <sub>9</sub>	F: CCAACAAACACGAGGAATGA R: CCACACTGATGTTCCAGCAC
5	Rw35C24	$(AG)_{9}X(AG)_{8}$	F: GGCGAATCGAGATTCAGAGA R: GGATTAGCCCAAGTCCAGGT
6	Rw34L6	(CT) <sub>16</sub>	F: CTCCTTTAGACTCGGGACCA R: CAGGCACGCCATTTCTAACT
7	Rw52D4	(TTC) <sub>7</sub>	F: GGCAGTTGCTGTGCAGTG R: TTGTGCCGACTCAAAATCAA
8	RA003a	(GA) <sub>30</sub>	F: CAGAATTGGGTGTCCGTATG R: CAATTTTCAAAGGATAATTTGG
9	RA013a	(AG) <sub>13</sub>	F: GAGGGGAAAGAGATACACAAA R: GTAAGACCTTGCGTGTTCATA
10	RA016a	(AG) <sub>21</sub>	F: CAGGTGAAGAAGAGAAGGGTGT R: CCTCAGTTCATTTCAATCATCTCC
11	RA019a	(AG) <sub>11</sub> (AC) <sub>9</sub>	F: CGTTAGAGATCCGAGGGGGGTC R: TGTCATGGTTGGGAAGTTGGCT
12	RA020a	(AG) <sub>15</sub> (AC) <sub>12</sub>	F: GTTAGAACCGAAGGCTCTAGT R: CCCGCTAAGGTGGAGACATAC
13	RA023b	(GA) <sub>20</sub>	F: CATCCTCGGTGTTGCGTTGA R: TGTCTCCAGCAACCTTTTTTTCCC
14	RA027a	(AG) <sub>25</sub> A(CAGAGA) <sub>5</sub>	F: ACCGTCCACAGTGTAAGAAAG R: CCCTCAAGTCTAGTAAAACCA
15	RA032b	(GA) <sub>23</sub>	F: CGGCATCAAAGATATAGCTTCC R: AGAAATGCAAAACGCCCCTATGA
16	RA034a	(GA) <sub>22</sub>	F: GCATAGAGAACTCGGGAATCAC R: TTCCGAAATGCCAACAACCAG
17	RA037a	(GA) <sub>21</sub>	F: AGAGAGTATGTCGTTTGGAGGAG R: CTGCCTAAAATACCCCAAGTCAT
18	RA042a	(GA) <sub>26</sub> GT(GA) <sub>8</sub>	F: CAGACTTATCAATGCGATCGTGCC R: CAGCAATTCAGCAAGCCGTCTC
19	RA043a	(AG) <sub>17</sub>	F: GCAACGTACTTCAATTTCCAC R: CAAGCTCAGAACTGAGACAC
20	RA044b	(AG) <sub>14</sub>	F: TAGACAGATAGATATTGGCAC R: CAACTACAGATTTCTACCAACT

Table 1. SSR primers used in the amplification of oil-bearing roses with their respective sequences.

No	Genotypes	Petal Color	Petal Number per Flower	DNA Quantity (ng μl <sup>-1</sup> )	DNA Quality (260/280)	Number of Successful SSR Primers		
1	Rd-M0-10*	Pink	40	19.4	2.15	19		
2	Rd-M0-19	Pink	30	60.1	1.96	20		
3	Rd-M0-29	Dark pink	5-10	56.7	1.90	19		
4	Rd-M0-31	Pink	45-50	54.6	1.95	19		
5	Rd-M0-37	Light pink	25-30	45.4	1.97	20		
6	Rd-M0-43	Pink	65	42.2	1.91	20		
7	Rd-M0-44	Light pink	20-25	65.6	1.92	18		
8	Rd-M0-49	Pink	25-30	48.1	1.98	20		
9	Rd-M0-51	Dark pink	25	29.3	1.95	20		
10	Rd-M0-52	White	60	32.7	1.96	19		
11	Rd-M0-57	Dark pink	5	30.2	1.96	19		
12	Rd-M0-60	Red	5	35.8	1.93	19		
13	Rd-M0-66	Pink	55-60	48.3	1.93	20		
14	Rd-M0-67	Light pink	30	49.5	1.94	20		
15	Rd-M0-70	Pink	30	31.2	1.92	16		
16	Rd-M0-76	Pink	40-45	14.9	2.40	20		
17	Rd-M0-77	Pink	55	37.3	1.99	20		
18	Rd-M100-15	Pink	35-40	23.3	2.12	18		
19	Rd-M100-18	White	35	75.3	1.92	20		
20	Rd-M100-28/1	White	60-65	95.4	1.93	20		
21	Rd-M100-29	Pink	25-30	74.6	1.96	15		
22	Rd-M100-33	Pink	20-25	24.2	1.97	-		
23	Rd-M100-37	Dark pink	35-40	54.8	1.93	19		
24	Rd-M100-44	Pink	50	6.40	1.90	-		
25	Rd-M100-55	Pink	35-40	54.2	1.91	20		

Table 2. Floral characteristics, DNA quantity and quality of the rose genotypes and PCR amplification of 20 SSR primers.



No	Genotypes	Petal Color	Petal Number per Flower	DNA Quantity (ng μl-1)	DNA Quality (260/280)	Number of Successful SSR Primers			
26	Rd-M100-68	Light pink	50	39.7	1.88	18			
27	Rd-M100-78	Pink	85-90	57.0	1.90	18			
28	Rd-M100-81	Light pink	25-30	34.5	2.04	20			
29	Rd-M100-98	Light pink	95-100	55.3	1.85	20			
30	Rd-M100-100	White	40-45	28.8	1.95	20			
31	Rd-M100-104	Pink	5	64.9	1.92	20			
32	Rd-M100-105	Pink	30-35	46.5	1.99	20			
33	Rd-M100-110	Light pink	60-65	47.7	1.99	19			
34	Rd-M100-111	Pink	5-6	65.2	1.94	19			
35	Rd-M100-116	Pink	25	19.4	2.15	19			
36	Rd-M200-4	Light pink	60	35.7	2.00	19			
37	Rd-M200-9	Pink	30-35	28.4	2.06	19			
38	Rd-M200-16	Dark pink	10	33.3	2.03	20			
39	Rd-M200-17	Light pink	20-25	36.6	1.96	19			
40	Rd-M200-18	Pink	30-35	78.1	1.92	18			
41	Rd-M200-21	Light pink	10	73.7	1.61	17			
42	Rd-M200-23	Dark pink	5-10	25.1	2.03	20			
43	Rd-M200-26	Light pink	25	81.9	1.90	19			
44	Rd-M200-28	Pink	5	47.1	1.95	19			
45	Rd-M200-31	Light pink	20	40.6	2.05	18			
46	Rd-M200-34	White	5	29.2	2.03	20			
47	Rd-M200-35	Pink	90	43.1	1.98	20			
48	R. damascena	Pink	30	35.6	1.90	20			

Continuing table 2

'Rd refers to Rosa damascena; M refers to mutation; 0 (control), 100 and 200 refer to gamma ray doses

		SSR Primers																		
Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
4	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
11	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+		+	+	+	+		+	+	+		+	+	+		+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21		+		+	+	+	+	+	+	+	+		+	+	+		+	+		+
23	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 3. PCR amplifications in Rosa damascena genotypes scanned with twenty different SSR primers.


Continuing table 3

Constras	SSR Primers																			
Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
26	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+
27	+		+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
31	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
34	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
36	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
38	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
39	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+		+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
41	+	+	+	+	+	+	+		+	+		+	+	+	+		+	+	+	+
42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
43	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
44	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+
45	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+	+	+	+
46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
47	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
48	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\*Empty (blue) areas indicate that amplification product has not occurred

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## Genetic Architecture of Morpho-Physiological Traits in Wheat Accessions under Terminal Heat Stress

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#### ABSTRACT

This study investigated genetic variability, character association and path analysis for 12 morphological and 6 physiological traits in fourty wheat accessions at CCS Haryana Agricultural University, Hisar during *rabi* 2018-19 growing season. The mean sum of squares due to genotypes were highly significant for all the morpho-physiological characters studied hereby indicates enough variability for selection of heat tolerant genotypes for further crop improvement. Phenotypic and genotypic coefficients of variation were recorded highest for grain yield followed by biological yield, peduncle length and 1000 grain weight, signifying scope for genetic improvement through selection. High heritability coupled with high genetic advance was reflected for grain yield, peduncle length, biological yield, 1000 grain weight and plant height. Correlation studies showed significant and positive association of grain yield with biological yield, 1000 grain weight, harvest index, SPAD 1, SPAD 2, grains per spike, spike length, peduncle length, NDVI 2, NDVI 1 and tillers per plant. Biological yield exerted the highest positive direct effect on grain yield followed by harvest index, SPAD1 and peduncle length. Hence, due emphasis should be given to these attributes for genetic improvement in wheat under heat stress condition.

Keywords: Genetic variability, correlation, path analysis, wheat, heat stress.

#### Introduction

Wheat (Triticum aestivum L), a self-pollinating annual plant in the true grass family Poaceae, is the largest cereal crop extensively grown as staple food in the world. It is one of the most important export and strategic cool-season cereal crop in the world in terms of production and utilization (Guin et al. 2019). Globally, India has the largest area under wheat cultivation and is the second largest producer after China. (USDA, 2017). Nationally, wheat is the second most important food crop after rice (Dey, 2020). It is cultivated extensively in North-Western and Central Zones. India has reported a record production of 101.20 million tonnes wheat from an area of 29.55 million hectare with a productivity of 3424 kg/ha during the crop season 2018-19. In Haryana, 11.65 million tonnes wheat was produced on an area of 2.51 million hectare with average productivity of 4643 kg/ha (ICAR-IIWBR, 2019).

In India, rice-wheat cropping system is spread over 11 million hectares in the Indo-Gangetic Plains and is important for national food security, but due to the long duration of basmati rice, sowing time of wheat in this region is pushed beyond the month of November. Wheat delayed sowing causes supra-optimal thermal stress at the reproductive phase (Preeti et al. 2016a). The prevalence of reproductive stage at heat stress has been found to be more detrimental in wheat production as compared to early heat stress due to its direct effect on grain number and grain weight (Nawaz et al. 2013). Low latitude zones, where around 100 million hectares of wheat is cultivated, are predominantly heat prone areas worldwide (Braun et al. 2010). In India the most significant impacts of high temperature are being experienced in the Gangetic plains zone in the form of shorter winters and the onset of significantly higher temperatures much earlier than normal (Chandra et al. 2017).

Therefore, heat can influence the different stages of crop growth during crop cultivation in Indo-Gangetic Plains (Preeti et al. 2016b).

To adapt new wheat varieties to the future climate change, we need to understand how they respond to elevated temperatures and how tolerance to heat can be improved (Halford 2009). Therefore, there is need to exploit the existing genetic variability in wheat for developing high yielding and good quality varieties under changing climatic scenario (Kant et al. 2014). Identification of genetically superior parents is an important pre-requisite for developing promising genotypes for effective transfer of targeted genes controlling both quantitative and qualitative traits in the resultant progenies. Thus, the estimation of genetic parameters like heritability and genetic advance is essential for a breeder which helps in understanding the magnitude, nature and interaction of genotype and environmental variation of the traits.

Correlation studies provide better understanding of yield component which helps the plant breeder during selection (Johnson et al. 1955). Path coefficient analysis measures the direct and indirect contribution of independent variables on dependent variables and thus helps breeder in determining the yield components and understanding cause of association between two variables. The information obtains by path coefficient analysis also helps in indirect selection for genetic improvement of yield because direct selection is not effective for low heritable trait like yield. Hence, the present investigation was carried out to evaluate genetic variability and to determine correlation coefficient and path analysis among morpho-physiological traits in wheat accessions under terminal heat stress conditions.

#### **Materials and Methods**

The present study was conducted at Research Area of Wheat and Barley Section of Department of Genetics & Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar located at an altitude of 215.2 meters above mean sea, latitude of 29° 10' N and at longitude of 75° 46' E. The experimental material consisted of 40 wheat accessions (Table 1) along with four check varieties namely WH 711, WH 542, WH 1124 and HD 3059 raised under optimum input conditions (150 kg N, 60 kg P<sub>2</sub>O<sub>5</sub> and 25 kg ZnSO<sub>4</sub> per hectare based on soil testing reports). These wheat accessions were received from National Bureau of Plant Genetic Resources (NBPGR), New Delhi for screening against terminal heat stress tolerance. The experiment was planted on 24th December under late sown condition in randomized block design (RBD) with 3 replications during rabi season of 2018-19. Each accession was sown in paired rows of 2.5 m length with inter-row and inter-plant distances of 20 and 10 cm, respectively. Observations were recorded at specific stage on five randomly selected plants per accession per replication for 12 morphological traits viz., 1) days to heading, 2) days to maturity, 3) grain filling duration, 4) plant height (cm), 5) peduncle length (cm), 6) tillers per plant, 7) spike length (cm), 8) grains per spike, 9) 1000 grain weight (g), 10) grain yield per plant (g), 11) biological yield per plant (g) and 12) harvest index (%) and 6 physiological traits viz., 1) normalized difference vegetation index at anthesis (NDVI 1), 2) normalized difference vegetation index at 15 days after anthesis (NDVI 2), 3) canopy temperature at anthesis (CT 1), 4) canopy temperature at 15 days after anthesis (CT 2), 5) soil plant chlorophyll development at anthesis (SPAD 1) and 6) soil plant chlorophyll development at 15 days after anthesis (SPAD 2).

The mean performance of each accession was recorded and employed for statistical analysis. Analysis of variance to test the significance for each character was carried out as per methodology advocated by Fisher (1925) and described by Panse and Sukhatme (1967). Phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) were calculated by the formula given by Burton (1952), heritability in broad sense (h<sup>2</sup>) was calculated by using the formula suggested by Hanson et al. (1956) and genetic advance that is the expected genetic gain was calculated by using the procedure given by (Johnson et al. 1955). Correlation and path coefficients were worked out as per method suggested by Al-Jibouri et al. (1958) and Dewey and Lu (1959), respectively.

#### **Results and Discussion**

The mean sum of squares due to genotypes were highly significant for all the morphological and physiological characters studied hereby indicating a wide range of allelic variability, which could be used for selection of heat tolerant genotypes for further crop improvement. The presence of significant variability has also been reported by Mansouri et al. (2018) and Suresh et al. (2018) for various morphological traits and for physiological traits by Sharma et al. (2018) and Sangwan et al. (2018).

Table 2 depicts the estimates of genetic variability parameters for all the characters. In general, the results revealed wide range for all the traits under investigation. Less difference in the estimates of genotypic and phenotypic variances depicted little influence of environment on the expression of traits studied. The estimates of genotypic and phenotypic coefficient of variation ranged from 2.99 to 24.42% and 3.63 to 26.20%, respectively. The perusal of data revealed highest phenotypic and genotypic coefficients of variation for grain yield followed by biological yield, peduncle length and 1000 grain weight, indicating availability of enough genetic variability and thus exhibited scope for genetic improvement through selection. However, days to heading and maturity exhibited least phenotypic and genotypic coefficients of variation. Similar findings were also reported by Veeresha and Naik (2016), Neeru et al. (2017), Mansouri et al. (2018) and Rathwa et al. (2018) in wheat.

Heritability determines the extent of genetic control of a given trait and its transmission to progeny and, hence has bearing on the selection efficiency of trait concerned. Heritability in broad sense was found to be maximum for 1000 grain weight (94.78%) followed by plant height (93.26%) and was recorded minimum for harvest index (62.32%). The estimates of heritability are more advantageous when expressed in terms of genetic advance. (Johnson et al. 1955) advocated consideration of heritability and genetic advance together for effective selection. High heritability coupled with high genetic advance reflected for grain yield, peduncle length, biological yield, 1000 grain weight and plant height, therefore, the variability present in these traits was of additive nature, which can be better utilized for crop improvement. These findings confirm with the results obtained by Kant et al. (2011), Neeru et al. (2017), Rathwa et al. (2018) and Suresh et al. (2018).

Correlation analysis was done separately for morphological and physiological traits, whose results have been presented in Table 3 and 4, respectively. The results of correlation analysis under heat stress condition revealed significant and positive association of grain yield with biological yield, 1000 grain weight, harvest index, grains per spike, spike length, peduncle length and tillers per plant; and significant negative association with days to heading and days to maturity. Significant positive correlation was also observed for days to heading with days to maturity; grain filling duration with days to maturity, plant height, peduncle length and biological yield; plant height with peduncle length, 1000 grain weight and biological yield; peduncle length with 1000 grain weight and biological yield; spike length with grains per spike and biological yield; grains per spike with 1000 grain weight, biological yield and harvest index; and 1000 grain weight with biological yield. Similar results



The estimates of correlation coefficients among different physiological traits and with grain yield are depicted in Table 4. The results showed significant positive correlation of grain yield with physiological traits viz., NDVI 1, NDVI 2, SPAD 1 and SPAD 2, however CT 1 and CT 2 exhibited significant negative correlation with grain yield. Similar results were also obtained by Lopes and Reynolds (2012) and Kumar et al. (2018). Mohammadi et al. (2012) and Mansouri et al. (2018) also recorded negative correlation between grain yield and canopy temperature. Significant positive correlation was observed for NDVI 1 with NDVI 2, SPAD 1 and SPAD 2; NDVI 2 with SPAD 1 and SPAD 2; CT 1 with CT 2; and SPAD 1 with SPAD 2. Similarly, significant negative correlation was recorded for NDVI 1 and NDVI 2 with CT 2 and CT 1; and CT 2 with SPAD 1 and SPAD 2.

The results of path coefficient analysis for morphological traits are presented in Table 5 and for physiological traits in Table 6. Biological yield (0.856) exerted the highest positive direct effect on grain yield followed by harvest index (0.459) and peduncle length (0.222). Similar results were also reported by (Islam et al. 2017) and (Suresh et al. 2018). The highest negative direct effect on grain yield was recorded for plant height (-0.287). Mohanty et al. (2016) and Suresh et al. (2018) also reported negative direct effect of plant height on grain yield, which support our finding. The results also showed maximum positive indirect effect of 1000 grain weight on grain yield through biological yield, whereas, plant height exhibited highest negative indirect effect via harvest index. The low residual effect (0.013) indicated that most of the variability in grain yield for the genotypes under study has been explained by the independent variables included in the analysis.

The path analysis for physiological characters revealed that the traits SPAD 1 had maximum direct effect on grain yield, followed by SPAD 2 and NDVI 1. These results are similar to the findings of Mądry et al. (2015), Neeru et al. (2017) and Khanal et al. (2020). The trait SPAD 2 had highest positive, while CT 2 recorded highest negative indirect effect on grain yield through SPAD 1. A residual factor of 0.749 depicted



that six physiological parameters used in the study were unable to account for a major portion of the variability present in grain yield. It also denotes that other possible independent variables which were not included in the study had a significant effect on grain yield. No value of direct effect exceeded one indicating that inflation due to multicollinearity was minimal. The traits *viz.*, biological yield, harvest index, peduncle length and SPAD 1 exhibited true positive association, while canopy temperature exhibited true negative association with grain yield as the values of their genotypic correlation coefficients and direct effect on grain yield are fairly close to each other. Consequently, it is suggested that these parameters can be considered as key components for wheat improvement under heat stress.

Sr. No.	Accessions	Accession No.	Sr. No.	Accessions	Accession No.
1	DT 5	IC 335583	21	DT 139	IC 335968
2	DT 25	IC 335966	22	DT 142	IC 111844
3	DT 46	EC 609336	23	DT 147	IC 445528
4	DT 54	EC 276983	24	DT 150	IC 535772
5	DT 83	IC 296756	25	DT 151	IC 535518
6	DT 101	IC 543401	26	DT 153	EC 276814
7	DT 102	EC 277323	27	DT 154	IC 543364
8	DT 104	EC 276920	28	DT 168	EC 276864
9	DT 106	IC 534137	29	DT 169	IC 547701
10	DT 109	IC 402058	30	DT 171	EC 295392
11	DT 110	IC 276717	31	DT 175	EC 478016
12	DT 113	IC 542124	32	DT 176	EC 299085
13	DT 114	EC 313735	33	DT 177	EC 573837
14	DT 116	EC 519498	34	DT 178	IC 128664
15	DT 122	EC 577722	35	DT 181	EC 577619
16	DT 124	IC 47337	36	DT 183	IC 535848
17	DT 125	EC 609337	37	DT 187	IC 445522
18	DT 126	EC 445157	38	DT 190	IC 335932
19	DT 127	EC 609574	39	DT 191	EC 519501
20	DT 137	IC 35143	40	DT 192	EC 13263
Check Variety 1	WH 711		Check Variety 3	WH 1124	
Check Variety 2	WH 542		Check Variety 4	HD 3059	

#### Table 1. List of wheat genotypes used in the study.

Traits	Mean ± SE	Range	GCV (%)	PCV (%)	Heritability (bs) (%)	Genetic Advance (% of mean)
$\mathbf{D}\mathbf{H}^{+}$	$83.93 \pm 1.77$	75.00 - 93.33	4.99	6.18	65.23	8.30
DM	$120.72\pm1.44$	115.00 - 129.67	2.99	3.63	67.76	5.06
GFD	$30.77 \pm 1.08$	22.67 - 37.67	8.81	10.70	67.79	14.95
РН	$104.59\pm2.71$	75.07 - 145.27	16.70	17.29	93.26	33.22
PL	$36.47 \pm 1.11$	21.90 - 49.90	18.70	19.43	92.59	37.06
SL	$10.81\pm0.33$	8.63 - 13.37	9.07	10.53	74.26	16.10
TIL	$7.25\pm0.27$	5.27 - 9.20	10.10	12.02	70.68	17.50
GPS	$53.68 \pm 1.35$	43.67 - 62.73	8.95	9.95	80.76	16.56
TGW	$32.98\pm0.78$	20.91 - 43.76	17.37	17.84	94.78	34.83
BY	$27.74 \pm 1.61$	16.97 - 36.90	19.51	21.94	79.09	35.75
GY	$8.59\pm0.47$	5.10 - 14.10	24.42	26.20	86.83	46.87
HI	$31.27\pm2.10$	18.13 - 41.95	14.96	18.95	62.32	24.33
NDVI 1	$0.75\pm0.02$	0.63 - 0.83	6.44	7.87	67.08	10.87
NDVI 2	$0.67\pm0.02$	0.50 - 0.74	7.36	8.47	75.55	13.18
CT 1	$24.65\pm0.67$	22.47 - 28.37	6.58	8.11	65.83	10.99
CT 2	$28.48\pm0.72$	25.03 - 31.23	5.68	7.18	62.41	9.24
SPAD 1	$44.55\pm1.93$	32.73 - 55.6	10.62	13.01	66.74	17.88
SPAD 2	$40.25\pm1.34$	30.30 - 49.13	9.09	10.77	71.24	15.81

Table 2. Genetic variability parameters for different morpho-physiological traits.

\*DH: Daysto heading (day), DM: Daysto maturity (day), GFD: Grain filling duration (day), PH: Plantheight (cm), PL: Pedunclelength (cm), SL: Spike length (cm), TIL: Tillers per plant, GPS: Grains per spike (%), TGW: 1000-grain weight (g), BY: Biological yield per plant (g), GY: Grain yield per plant (g), HI: Harvest index (%), NDVI1: Normalized difference vegetation index at anthesis, NDVI 2: Normalized difference vegetation index at 15 days after anthesis, CT 1: Canopy temperature at anthesis, CT 2: Canopy temperature at 15 days after anthesis, SPAD 1: Soil plant chlorophyll development at anthesis, SPAD 2: Soil plant chlorophyll development at 15 days after



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Traits	DH	DM	GFD	РН	PL	SL	TIL	GPS	TGW	BY	HI	GY
$\mathbf{D}\mathbf{H}^{+}$	1.000	0.630**	-0.402**	-0.307**	-0.431**	-0.077	-0.211*	0.092	-0.097	-0.297**	0.001	-0.274**
DM		1.000	0.544**	-0.068	-0.045	-0.172*	-0.291**	0.002	-0.008	-0.170	-0.029	-0.188*
GFD			1.000	0.239**	0.327**	-0.068	0.066	-0.060	0.134	0.225**	-0.049	0.135
РН				1.000	0.824**	-0.106	0.086	-0.120	0.491**	0.583**	-0.667**	0.088
PL					1.000	0.075	0.003	-0.257**	0.526**	0.537**	-0.282**	0.288**
SL						1.000	-0.118	0.503**	0.160	0.296**	0.046	0.323**
TIL							1.000	-0.102	0.042	0.163	0.143	0.213*
GPS								1.000	0.291**	0.322**	0.217*	0.414**
TGW									1.000	0.784**	0.100	0.719**
BY										1.000	-0.106	0.789**
HI											1.000	0.517**
GY												1.000

Table 3. Genotypic correlation coefficients among different morphological traits in wheat genotypes.

\*, \*\* Significant at 5% and 1% level, respectively

\*DH: Daystoheading(day), DM: Daystomaturity(day), GFD: Grainfillingduration(day), PH: Plantheight(cm), PL: Pedunclelength(cm), SL: Spike length (cm), TIL: Tillers per plant, GPS: Grains per spike (%), TGW: 1000-grain weight (g), BY: Biological yield per plant (g), GY: Grain yield per plant (g), HI: Harvest index (%)

Table 4. Genotypic correlation coefficients among different physiological traits and grain yield in wheat genotypes.

Physiological Traits	NDVI 1	NDVI 2	<b>CT 1</b>	CT 2	SPAD 1	SPAD 2	GY
NDVI 1 <sup>+</sup>	1.000	0.698**	-0.07	-0.561**	0.369**	0.427**	0.274**
NDVI 2		1.0001	-0.157	-0.316**	0.514**	0.421**	0.285**
<b>CT 1</b>			1.000	0.214*	-0.265**	-0.221*	-0.200*
CT 2				1.000	-0.478**	-0.418**	-0.364**
SPAD 1					1.000	0.862**	0.467**
SPAD 2						1.000	0.415**

\*, \*\*Significant at 5% and 1% level, respectively

\*NDVI 1: Normalized difference vegetation index at anthesis, NDVI 2: Normalized difference vegetation index at 15 days after anthesis, CT 1: Canopy temperature at anthesis, CT 2: Canopy temperature at 15 days after anthesis, SPAD 1: Soil plant chlorophyll development at anthesis, SPAD 2: Soil plant chlorophyll development at 15 days after anthesis

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Table 5. Direct (diagonal values) and indirect effects of different morphological traits on grain yield in wheat genotypes.

Traits	DH	DM	GFD	РН	PL	SL	TIL	GPS	TGW	BY	HI	Correlation with GY
$\mathbf{D}\mathbf{H}^{+}$	0.057	-0.062	-0.015	0.088	-0.096	0.004	-0.003	0.008	-0.001	-0.254	0.000	-0.274**
DM	0.036	-0.098	0.020	0.020	-0.010	0.009	-0.004	0.000	0.000	-0.146	-0.013	-0.188*
GFD	-0.023	-0.054	0.036	-0.069	0.073	0.003	0.001	-0.005	0.001	0.193	-0.023	0.135
РН	-0.017	0.007	0.009	-0.287	0.183	0.005	0.001	-0.010	0.004	0.499	-0.306	0.088
PL	-0.024	0.005	0.012	-0.236	0.222	-0.004	0.000	-0.021	0.005	0.460	-0.130	0.288**
SL	-0.004	0.017	-0.002	0.031	0.017	-0.049	-0.002	0.041	0.001	0.253	0.021	0.323**
TIL	-0.012	0.029	0.002	-0.025	0.001	0.006	0.014	-0.008	0.000	0.140	0.066	0.213*
GPS	0.005	0.000	-0.002	0.034	-0.057	-0.025	-0.001	0.081	0.003	0.276	0.100	0.414**
TGW	-0.006	0.001	0.005	-0.141	0.117	-0.008	0.001	0.024	0.009	0.672	0.046	0.719**
BY	-0.017	0.017	0.008	-0.167	0.119	-0.015	0.002	0.026	0.007	0.856	-0.049	0.789**
HI	0.000	0.003	-0.002	0.191	-0.063	-0.002	0.002	0.018	0.001	-0.091	0.459	0.517**

**Residual factor = 0.0135** 

\*, \*\* Significant at 5% and 1% level, respectively

\*DH: Daystoheading(day), DM: Daystomaturity(day), GFD: Grain filling duration(day), PH: Plantheight(cm), PL: Pedunclelength(cm), SL: Spike length (cm), TIL: Tillers per plant, GPS: Grains per spike (%), TGW: 1000-grain weight (g), BY: Biological yield per plant (g), GY: Grain yield per plant (g), HI: Harvest index (%)

Table 6. Direct (diagonal values) and indirect effects of different physiological traits on grain yield in wheat genotypes.

Physiological Traits	NDVI 1	NDVI 2	<b>CT 1</b>	CT 2	SPAD 1	SPAD 2	Correlation with GY
NDVI 1 <sup>+</sup>	0.032	0.016	0.005	0.088	0.119	0.015	0.274**
NDVI 2	0.022	0.023	0.011	0.049	0.165	0.014	0.285**
CT 1	-0.002	-0.004	-0.068	-0.033	-0.085	-0.008	-0.148
CT 2	-0.018	-0.007	-0.015	-0.156	-0.154	-0.014	-0.364**
SPAD 1	0.012	0.012	0.018	0.075	0.322	0.030	0.467**
SPAD 2	0.014	0.010	0.015	0.065	0.277	0.034	0.415**

**Residual factor = 0.7499** 

\*, \*\* Significant at 5% and 1% level, respectively

\*NDVI 1: Normalized difference vegetation index at anthesis, NDVI 2: Normalized difference vegetation index at 15 days after anthesis, CT 1: Canopy temperature at anthesis, CT 2: Canopy temperature at 15 days after anthesis, SPAD 1: Soil plant chlorophyll development at anthesis, SPAD 2: Soil plant chlorophyll development at 15 days after anthesis



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# Efficacy of Protein Hydrolysate (Plant Force Advance) Based Formulation on Cotton Yield

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#### ABSTRACT

A field experiment was conducted using Bt. hybrid cotton (var. RS2013) in Sri Ganganagar district of Rajasthan during Kharif 2017. The agronomic and biological parameters were studied in the cotton crop grown using protein hydrolysate (Plant Force Advance) from waste human hair. The test plots were given the foliar spray of liquid formulation (having approx. 8% (v/v) nitrogen and diluted 1:200 with water) after 25 days of seed germination followed by three consecutive sprays after interval of 30 days. The comparisons of means showed increase in height of the treated plants by 20.46%, enhancement in the chlorophyll content of plant leaves by 16.32%, increase in weight of balls per plant by 19.21% as well as 14.32% reduction in immature ball formation per plant as compared to control and the total yield showed an increase of 13.63%. The study concluded that the foliar application of protein hydrolysate along with recommended package of practices in Bt. hybrid cotton have promising results on the yield and growth of cotton under the field conditions.

Keywords: Bt. hybrid cotton, protein hydrolysate, amino acid-based bio-fertilizer.

#### Introduction

Cotton is the most important fibre crop of India and has the largest area under cotton cultivation in the world. Bt. hybrids constitute 87 per cent worldwide with increased yield of 8-10% till the release of Bollgard II (Sudha et al. 2011). The present hybrids though high yielding but are susceptible to pests like boll worms and number of viral infestations transmitted by the whitefly. Currently it is grown over 6 per cent of the net sown area and the coverage under Bt. hybrids in India is almost saturated and further improvement in cotton yield is not possible (Rao and Alapati, 2007) and presently the agronomists and cotton breeders are suggesting an alternative strategy to optimize cotton productivity by reducing production costs. The availability of most suitable cultivars, more efficient options of weed, pest and

disease management to modify morpho physiological frame, planting/harvesting tools has rekindled an interest in developing new types of fertilizer and exploring novel application patterns to ensure high fertilizer-use efficiency.

The increased crop production largely relies on the type of fertilizers used to supplement essential nutrients for plants, which has also led to over exploitation of chemical fertilizers and emerging environmental issues. So, there is a dire need to switch to natural biological based organic inputs as an alternative to agro-chemicals and the search to explore the cheap waste materials as new resources. Amino acid-based bio-fertilizers are gaining the high input in agricultural market because the formulation of amino acid bio-fertilizers are cheaper than the chemical fertilizers. Amino acids are fundamental ingredients in the process of protein synthesis and studies have proved that amino acids can directly or indirectly influence the physiological activities of the plant (Mostafa et al. 2014). The amino acidbased bio-fertilizers also act as bio-stimulant and were reported to promote plant growth by increasing the nutrient uptake efficiency of plant; tolerance to bear the biotic and abiotic stress; improve soil quality (Calvo et al. 2014); stimulate the development of roots and leaves (Popko et al. 2018). This approach has led to utilization of human hairs to produce a liquid amino acid-based bio-fertilizer that is very useful for every crop and do not cause single hazard to environment.

#### **Materials and Methods**

The experiment was carried out using Bt. hybrid cotton (var. RS2013) in the Research Farm of Surender Kaur Memorial Agricultural College, Padampur, Sri Ganganagar district of Rajasthan during Kharif 2017. The farm is geographically situated at an altitude of 165 m above mean sea level at 29.70°N latitude 73.62°E longitude and average temperature in summer reaches around 41°C and in winter around 26°C. The temperature shots up to 50°C in June. The average annual rainfall is only 200 mm per annum and the soil was sandy loam. The approved package of practices of cotton for Rajasthan State, Department of Agriculture was followed and the agronomic and biological parameters were studied in the cotton crop grown in one acre. The land preparation was done with one deep ploughing followed by 2-3 harrows. Sowing was done in the month of June and seed rate was 16.0 kg/ha. In sowing method, the row to row spacing is 67.5 cm, plant to plant spacing is 30 cm and sowing depth was about 4-5 cm. The thinning was done 25-30 DAS to maintain plant to plant distance of 30 cm. The fertilizers used were Nitrogen 80 kg/ ha, Phosphorus 40 kg/ha and Potash 20 kg/ha and ZnSO<sub>4</sub> 12 kg/ha. The experiment was carried out in randomized plot design with foliar spray as main treatment and control. The liquid formulation "Plant Force Advance" of Floritech Organo Industries, Nagpur was tested for its efficacy in the experiment. The test plots were given the foliar spray of liquid formulation (having approx. 8% (v/v) nitrogen and diluted 1:200 with water) 25 days after germination of the seeds followed by three more sprays each after the interval of 30 days. The colorimetric estimation of chlorophyll content in leaves at various stages of growth i.e square stage, flowering and boll initiation was performed by the method of Arnon (1949).

At harvest stage, the five randomly selected plants were taken from the treatment and control separately



to record the observations on yield parameters in terms of seed yield plant<sup>-1</sup>, boll weight and immature boll formation (%), the number of bolls per plant. The results were statistically analysed using Duncan's multiple range test.

#### **Results and Discussion**

The efficacy of protein hydrolysate (Plant Force Advance) formulation on yield and growth parameters of Bt. hybrid cotton (var. RS2013) was investigated under field conditions. Plant height is an important morphological character in cotton which provides seat for nodes and internodes from where monopodial and sympodial branches emerge and thus play an important role in determining morphological framework relating to productivity (Eaton 1955). Plant height was recorded at 40, 60 and 90 DAS representing square, flower and boll initiation stages of the crop. Height of plants treated with foliar spray of protein hydrolysate was found significantly higher than the control plants. The comparisons of means showed increase in height of the treated plants by 20.46% at all the three stages as compared to control (Table 1). Chlorophyll is the pigment primarily responsible for photosynthesis. It absorbs energy from sunlight and helps converts it into chemical energy during the light dependent reactions of photosynthesis. Chlorophyll determines the photosynthetic capacity and influence the rate of photosynthesis, dry matter product and yield (Gitelson 2003). The enhancement in the chlorophyll content of plant leaves treated with foliar spray of protein hydrolysate was found by 16.32% as compared to control (Table 1). The results suggested that the protein hydrolysate contains free amino acids and low molecular weight short peptides, which act as organic nitrogen source used for synthesis of macromolecules like chlorophyll (Subbarao et al. 2015).

The results showed that maximum boll weight was 2.42 g as compared to control plot. The increase in weight of balls per plant was 19.21% as well as 14.32% reduction in immature ball formation per plant in protein hydrolysate treated plants as compared to control. The overall total yield showed an increase of 13.63% in the test plot over the control (Table 2).

Several experimental studies testing the action of protein hydrolysate under both open-field and controlled conditions, have demonstrated that they stimulate shoot and root biomass, resulting in increased productivity of several crops such as corn, kiwifruit, lettuce, lily, papaya, passion fruit, pepper and tomato (Schiavon et al. 2008; Ertani et al. 2009; Colla et al. 2014, 2015, 2017; Halpern et al. 2015; Nardi et al. 2016). Foliar application of animal and plant-derived protein hydrolysate has also been shown to promote the vegetative growth and yield of several fruit trees (Colla et al. 2015).

In the present study the foliar application of plant force represents a new range of biofertilizers obtained from human hairs as hydrolysate having short chain amino acids along with other peptides can easily assimilated by plant tissues. From earlier studies it is evident that a nitrogen nutrition enhances both plant growth as well as development due to improved overall metabolism and nutrient supply (Laghari et al. 2016). This innovative product possesses high amount of organic nitrogen and hence may be important element of organic agriculture. The application of protein hydrolysate stimulated an auxin like activity due to the presence of tryptophan and some peptides and could be of practical interest for promoting the growth of plants and their great efficacy in enhancing crop performances. The foliar and root applications of protein hydrolysate could exhibit hormone-like activities (specifically auxin and gibberellin-like activity) leading to stimulation of seed germination, plant growth, fruit set and enlargement (Yadav and Khosla 2020).

These results agreed with the reports of other researchers who evaluated the effects of amino acids on the yield and/or growth of common bean, two wheat cultivars and *Urtica pilulifera* plants and suggested that foliar application of amino acid liquid fertilizer showed agreeable results (Moreira et al. 2017; El-Said and Mahdy 2016; Wahba et al. 2015). However, previously reported amino acid liquid fertilizers were produced by one or a solution composed of different amino acids, whereas the amino acids used in the present study were created from human hairs resulted from the salons and local municipal waste. Due to the improvement of urban living standards, the increasing development of municipal waste and problem of choking with human hairs as non-degradable waste, especially for the scale causing a huge amount of animal hair waste in the city's sewage systems that can generate a great risk to the environment. Thus, this study provides an effective and ecological leaf fertilization method based on amino acids created from human hairs that will not only enhance crop yield but also make full use of human hairs to protect the environment. Plant force is a good complement to both inorganic fertilisers and microbial based biofertilizers. Since it can metabolise directly, it gives quick response in growth which is reflected by stably enhanced chlorophyll content of the leaf. Its mimic urea fertilisation as it provides organic nitrogen almost in the same manner. Since nutrient used efficiency is strongly determined by host genotype therefore the present study should be extended to more number of cotton genotypes and other crops to identify the most potential combinations of varietal genotypes and plant force doses. This work is continued to achieve better insights of future for novel biofertilizers applications towards sustainable agriculture.

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	Square Stage		Flowerin	ng Stage	<b>Boll Initiation Stage</b>		
	Control	Treatment	Control	Treatment	Control	Treatment	
Height (cm)	24.75±1.29	29.83±1.91	38.02±6.17	45.8±7.44	51.74±5.17	62.33±6.23	
Chlorophyll (mg/g)	1.62±0.49	1.89±0.59	1.68±0.54	1.96±0.65	1.96±0.60	2.28±0.70	

Table 1. The growth and physiological attributes of cotton plants during the different growth stages of crop.

Table 2. The yield attributes during the two stages of boll initiation and boll burst stage of crop growth.

	Boll Initia	tion Stage	Peak Boll	Burst Stage
	Control	Treatment	Control	Treatment
Seed Yield (g) per plant	14.59±3.92	16.58±4.47	9.26±5.33	10.52±6.05
Boll weight (g)			2.03±0.31	2.42±0.35
Immature Boll formation (% decrease)			6.84±0.17	5.86±0.12

Figure 1. The photograph of cotton plants in field at mature harvest stage at SKM Agriculture College Research Farm, Padampur. (Original)





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### Development of Plant Growth Promoter Based on Globally and Abundantly Available Waste Banana Pseudo-Stem and Hair

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#### ABSTRACT

Banana is cultivated in more than 120 countries, over an area of 4.8 million hectares, with an annual production of 99.99 million tons in the year 2011 whereas in India alone in about 1.0 million hectares generating approximately 51.18 million tons of pseudo-stem as waste annually. Similarly, millions tones of small hair (approximately 2500-3000 MT) are being generated annually in India as waste. Both the waste material is abundantly, economically and easily available at throw away price. These waste materials contain various nutrients, vitamins, nitrogen, phosphorus Potassium and trace elements etc. useful for plant growth. With a view to develop wealth from these wastes namely banana pseudo-stem and hair, the study was carried out to develop plant growth promoter utilizing these wastes. Banana pseudo-stem juice (BPSJ) was extracted from the leaf sheath and tender core with the help of a juice extraction machine by squeezing pseudo-stem twice to extract the juice maximum. Plant growth promoter was prepared in laboratory using waste hair and banana pseudo-stem juice under acidic condition which contains dissolved solid (40%), amino nitrogen (4%), total nitrogen (7%), organic carbon (8%), total amino acids mixture (13%) and trace elements. The efficacy study of this product was carried out 5 L diluted in 200 L of water per acre on banana plantation. Drenching of this product was done five times at an interval of 1 month. During efficacy trials, parameters such as chlorophyll content, total biomass, root and shoot biomass and harvested fruit yield were studied. The efficacy reveals, increase in chlorophyll content (50%), total biomass (35%), root and shoot (50%) and fruit yield (15%) as compare to control. Based on efficacy study it is concluded that plant growth promoter has shown encouraging fruit growth and can be promoted among banana cultivators to enhance the productivity of crop.

Keywords: Banana, hair, hydrolysate, bio-efficacy, biomass.

#### Introduction

Banana is an herbaceous plant of the family Musaceae. In terms of overall production, it is in the second place after citrus, accounting for about 16% of the world's total fruit production (Deharveng et al. 1999). It is cultivated in more than 120 countries, over an area of 4.8 million hectares, with an annual production of 99.99 million tons in the year 2011 whereas in India alone in about 1.0 million hectares generating approximately 51.18 million tons of pseudo-stem as waste annually (Table 1) (Indian Horticulture Database, 2011). This crop generates a large amount of residue, due to the fact that each plant produces only one bunch of bananas. After the harvest, pseudo-stem is cut and usually left on the plantation site or burned, which could ultimately cause environment issues (Cordeiro et al. 2004). Thus, the utilization of the banana waste pseudo-stems has gained more attention in recent years. The banana pseudo-stem has been used as material for paper, furniture and forage (Buragohain et al. 2010; Umaz et al. 2005). Moreover, it has been also reported that these banana waste materials are rich in micronutrients, especially Mg, Ca, K, etc. (Aziz et al. 2011). The exploitation of waste banana pseudo-stems into value added products could significantly benefit to the society and increase its economic value.

Similarly millions tones of small hair (approximately 2500-3000 MT) are being generated annually in India as waste. Human hair is a material considered useless in the societies and therefore is found in the municipal waste streams in almost all cities and towns of the world (Kumar et al. 2009). The hair is thrown away in nature where it often accumulates in large amounts in the solid waste streams and chokes the drainage systems, posing a multifaceted problem. Due to slow degradation, it stays in the dumps/waste streams for long, occupying large volumes of space. Over time, leachate from these dumps increases the nitrogen concentration in the water bodies, causing problems of eutrophication. Burning of human hair or the waste piles containing them-a practice observed in many parts of the world-produces foul odor and toxic gases such as ammonia, carbonyl sulphides, hydrogen sulphides, sulphur dioxide, phenols, nitriles, pyrroles and pyridines (Brebu et al. 2012). Open dumps of hair generate hair dust which causes discomfort to people near them and if inhaled in large amounts, can result in several respiratory problems. Oils, sweat and other organic matter sticking to the hair rot over time and become a source of foul odor and breeding ground for pathogens (Ankush 2014).

To overcome these problems an effort was made to develop a system to utilize these waste materials as a resource. This study focused on efficient utilization of banana pseudo-stem and waste human hair to develop cost-effective and eco-friendly plant growth promoter.

#### **Materials and Methods**

Fresh pseudo-stem of banana was procured from the banana plantation field, Wardha, Maharashtra (India). The chemicals and media used were procured from Merck and Qualigens.

#### Extraction of banana pseudo-stem juice (BPSJ)

A fresh pseudo-stem of post-harvest banana plant was taken and the leaf sheaths and tender core (floral stalk) were manually separated from the pseudo-stem. The separated leaf sheaths and tender core were washed in running tap water. Juice was extracted from the leaf sheath and tender core with the help of a sugar-cane juice extractor machine. Fresh pseudo-stem banana plant juice was hazy, it represents 70% of pseudo-stem weight. The juice becomes brown after 2 hrs when exposed to air and light.

## Banana pseudo-stem juice hair hydrolysate (BPSJHH)

Banana pseudo-stem juice hair hydrolysate was prepared in laboratory using banana pseudo-stem juice and waste hair. The analysis of hydolysate shows dissolved solid (40%), amino nitrogen (4%), total nitrogen (7%), organic carbon (8%), total amino acids mixture (13%) and trace elements.

#### **Bio-efficacy studies on banana plants**

The bio-efficacy of banana pseudo-stem juice hair hydrolysate was carried out on banana plants in earthen pots at initial stage, in duplicate. For this purpose, 10 earthen pots, each pot (50x50x50 cm) was filled with soil up to 40 cm height and one rhizome of equal weight was put in the center and fully covered with soil. These pots were irrigated twice every week with 1 liter tap water.

When the banana plants were 5-week-old, first drenched with BPSJHH was made. Followed by the second, third, fourth and fifth drenching at one month interval (i.e. totally 5 times) of 2.5% volume/volume diluted in tap water. Total one liter was drenched in each pot whereas control was drenched with one liter of tap water.

The plants height, stem diameter was monitored at monthly interval for 6 months and Chlorophyll content was measured every month during the experimentation. After 6 months the experiment was terminated, observation was made for dry root biomass, shoot height, chlorophyll content and total biomass.

#### **Field trials**

After getting encouraging results from the above pot experiments, field trials on 50 Banana plants in duplicates was conducted in a black cotton soil. For this purpose, 5 liter BPSJHH thoroughly mixed with 200 liter of water, first drenched was made on 5 week old banana plants, followed by the second, third, fourth and fifth drenching at one month interval (i.e. totally 5 times).

#### **Results and Discussion**

## Banana pseudo-stem juice hair hydrolysate (BPSJHH)

Human Hair waste was hydrolyzed through chemical approach (5N HCL,100°C temp. 5 hrs) as it was rapid and cost-effective reproducible hydrolysate with more than 95% recovery. Hair, being a protein rich and banana pseudo-stem juice rich in micronutrients which is economically available raw material provided cost effective hydrolysate. Its rapid and easier processing afforded higher yield over the amino acids-based plant growth promoter available indigenously or from overseas. Due to absence of starch, it provided less caramelization and the resultant de-colourization, increased the recovery. Thus, techno economically banana pseudo-stem juice and waste hair appeared to be a superior potential raw material.

#### **Increased biomass**

Drenching of BPSJHH into the banana plants reproducibly gave 48% increase in the biomass and 50% increase in the chlorophyll content, 20% increase in the rhizome weight over the control plants (Table 2). The increase in biomass in each plant varied, per pot or plot, in terms of absolute values. However, the trend in biomass yield was always higher in different experimental pots or plots over their respective control. Therefore, it was desirable to accord an emphasis on the trend rather than percentage increase in absolute terms. These observations prompted us to carry out the BPSJHH drenching on large scale field trials.

#### **Eco-friendly aspects of PSBJHH**

The PSBJHH drenching did not affect the microbial flora qualitatively and if at all there was a change, it was a marginal increased due to highly nutritious, being a natural product and vital ingredient in different systems of living organisms. Therefore, PSBJHH is safe and eco-friendly products. From the above studies, it may he concluded that banana pseudo-stem juice and waste hair can be used for productive purpose waste to wealth. These wastes create problems of chocking the drainage pipes and thereby creating recurring environmental problems. Its hydrolysate, a plant tonic, could preserve ecology and enrich day to day nutrition by preserving soil microflora and creating greener environment/more food.

The beneficial attribute could be generation of plant growth stimulants may be due bio available of

organic compounds like amino acids mixture, organic carbon and trace elements availability to the roots provided more nutrient to the plant resulting in overall development of the plant.

Another possibility is that BPSJHH can act as nutrient for other beneficial microorganism to the plants and we have also observed plant vigour increased as compare to control. It may be attributed due to available of higher potassium and phosphorous which has contributed higher bio mass of banana pseudo-stem juice as well as biological yield.

The growth promoting effects of BPSJHH was evident with field trial at large scale which provided similar results as the earthen pot. Studies in field had the advantage of testing the response of plants under their normal condition of growth for agronomic purpose. We tested the response of the plants from germination to harvest and it provided 20% higher biological yield as compare to control.

The long-term effect of BPSJHH may contribute in improving the soil health, texture, microbial flora and may reduce the use of synthetic fertilizers. Therefore, its further evaluation on other commercially valuable crops and its long term economic and environmental effect may be carried to conclude its applicability in agriculture for improving the biological yield.

From the above studies, it could be concluded that Banana pseudo-stems juice and waste hair can be used for as a plant nutrients to increase plant productivity, instead of throwing as garbage.



Rank	<b>Country Production</b>	Millions of Tones
1	India	29.7
2	Uganda	11.1
3	China	10.7
4	Philippines	9.2
5	Ecuador	8.0
6	Brazil	7.3
7	Indonesia	6.1
8	Colombia	5.1
9	Cameroon	4.8
10	Tanzania	3.9
11	Australia	0.2

Table 1. Top 11 producers of bananas in the world andthe production of different countries in 2012.

Table 2.	Average chlorophyll, height, root biomass and
	shoot biomass and rhizome profile of banana
	plants.

Parameters Monitored	% Increase Over Control
Chlorophyll	50.00
Shoot height	20.00
Root biomass	35.00
Shoot biomass	48.00
Rhizome weight	20.00

Source: Horticulture Australia (HAL, 2012) FAO, 2011

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# Physiological Parameters of Bread Wheat (*Triticum aestivum* L.) Genotypes and Association with Yield and Quality under Rainfed Conditions

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#### ABSTRACT

Bread wheat is grown under a wide range of environmental conditions where climatic factors combined with agronomic inputs impart diverse effects on plant growth. This research was carried out in an experimental area of the Institute in Edirne, Trakya region (Turkey) to compare the yield, quality and some physiological parameters of wheat genotypes. The research was carried out with 11 genotypes in RCBD with 4 replications during 2012-2013 and 2013-2014 growing seasons. Data on grain yield, days to heading, plant height, 1000-kernels weight, test weight, biomass, canopy temperature and chlorophyll content were examined. According to the results, significant differences among genotypes in terms of yield, plant height, biomass, chlorophyll content and 1000-kernel weight were found. Genotypes G16-2012 and Tekirdağ had higher grain yield. Tekirdağ had the highest biomass and Bereket and Selimiye had higher chlorophyll content. The lowest canopy temperature was measured in genotypes G19-2012, Bereket, Selimiye and Aldane. The highest 1000-kernels weight and test weight were determined in cultivar Pehlivan and Selimiye. A positive correlation was determined between grain yield. Biomass in genotypes negatively correlated with days of heading, plant height, 1000-kernel weight and test weight and chlorophyll content. There was a negative correlation between canopy temperatures with grain yield. Biomass in genotypes negatively correlated with days of heading, plant height, 1000-kernel weight and test weight and chlorophyll content and biomass and a negative relation with canopy temperature, physiological parameters could be used in selection in bread wheat for yield potential under rainfed condition.

Keywords: Bread wheat, genotypes, yield, quality traits, physiological parameters.

#### Introduction

Wheat is one of the most important cereal crops in the world. As it is the major crop commodity for food security, there is a need to increase wheat production by developing new high yielding and climate-resilient varieties to meet the projected demand of increasing population and with changing climate (Khatodia et al. 2019). Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world due to its adaptation to diverse environments from -35 °C in the vegetative stage to 40 °C during grain filling period (Shewry, 2009). Genetic improvement in wheat yields in dry areas has not been as easy as in more favorable environments or where water is not a limiting factor (Richards et al. 2001). Breeding for stress tolerance/ resistance requires an assessment of the differential sensibility of relevant genotypes (Kant et al. 2014). It is only when the response of a genotype to given stress is known that more detailed analyses of the underlying physiological and/or genetic mechanisms of adaptation to stress can be undertaken. However, responses to abiotic stresses are more frequently quantitative than qualitative and, therefore, this task is not trivial. Stress characterization is often addressed as a particular case of genotype by environment interaction (GE). G×E is one of the statistical areas more extensively reviewed in plant breeding (Cooper and Hammer 1996; Annicchiarico 2002; Voltas et al. 2002; Romagosa et al.

2009). Plants can experience abiotic stresses resulting from the shortage of an essential resource or from the excess of a toxic substance or from climatic extremes. Occurrence, severity, timing and duration of stresses vary from location to location and in the same location from year to year. Furthermore, abiotic stress seldom occurs alone, the plants often face growing conditions characterized by a combination of different physical stresses (Cattivelli et al. 2002). Grain yield is a product of an organized interplay of its several components, which are highly susceptible to environmental fluctuations. However, yield can be estimated based on the performance of yield components. Enhancement in yield in most situations is more effectively fulfilled based on the performance of yield components, which are closely associated with grain yield (Ashfaq et al. 2003; Kant et al. 2011).

The use of infrared imaging to quantify the differences in the CT of wheat genotypes under drought was first reported by Blum and co-workers in 1982 and has also been shown to be an excellent predictor of yield in hot, irrigated environments (Reynolds et al. 1994). The trait was shown to explain approximately 60% of yield variation in Random Inbred Lines (RILs) under drought stress and is applied as a selection tool by breeders working in heat and drought-stressed environments (Trethowan and Reynolds 2007). Canopy temperature effected by biological and environmental factors like water status of soil, wind, evapotranspiration, cloudiness, conduction systems, plant metabolism, air temperature, relative humidity and continuous radiation (Reynolds et al. 2001), has preferably been measured at high air temperature and low relative humidity because of high vapour pressure deficit conditions (Amani et al. 1996). Phenotypic correlations of CT with grain yield were occasionally positive (Reynolds et al. 1994). The Normalized Difference Vegetation Index (NDVI) has frequently been used to evaluate the status of the crop and associate it with growth traits and grain yield (Morgunov et al. 2014). NDVI has also been shown to have a positive relationship with grain yield and biomass under wellirrigated conditions and a stronger association with yield under drought conditions (Reynolds et al. 1994; Gutierrez-Rodriguez et al. 2004; Marti et al. 2007). Genotypes with the horizontal orientation of leaves at the stem elongation stage had higher NDVI values compared to erect types and it was also determined that wheat yield would be more accurately predicted if NDVI was measured at both the early heading and the filling stage (Feng and Yang 2011). Moisture deficit differentially and significantly affected cultivar test weight and yield. The overall moisture-deficit-induced reduction in yield was primarily due to a reduction in kernel weight; effects of moisture deficit on a yield of specific cultivars were largely due to effects on kernels per spike. Cultivar x moisture treatment interactions was highly significant for test weight and yield (Guttieri et al. 2001). The main objective of this study was to investigate yield, physiological parameters and some agronomic characters of the genotypes under rainfed conditions.

#### **Materials and Methods**

The experiment was conducted in Edirne, Trakya region (Turkey) during the 2012-2013 and 2013-2014 growing seasons. This research was carried out with eleven genotypes. A randomized complete block design (RCBD) with four replications was used at each location. Each plot had 6 meter long, 6 rows, spaced 0.17 meter apart. Using a plot drill performed sowing and a seed rate of 500 seeds m<sup>2</sup> were used. Sowings were performed by using a plot drill in October and nitrogen was applied three times. Data on grain yield, plant height, days to heading, thousand kernels weights and test weight (Köksel et al. 2000) were investigated. In the research; physiological characters such as canopy temperature (CT), chlorophyll content (SPAD) and biomass (NDVI) were measured at the heading stage of the plant development.

A handheld portable SPAD-502 chlorophyll meter (Minolta) was used to estimate chlorophyll content (SPAD). This instrument provides a convenient means of assessing relative leaf chlorophyll content. Ten flag leaves were used to take chlorophyll meter readings from each plot at the heading stage (Z55). Chlorophyll meter data were taken on the same day or the closest possible day coinciding with the spectral reflectance measurements (Adamsen et al. 1999; Babar et al. 2006). A handheld infrared thermometer, with a field view of 2.5°C, was used to measure CT (°C). The data were taken from the same side of each plot at 1m distance from the edge and approximately 50cm above the canopy at an angle of 30°C to the horizontal. Readings were made between 13.00 and 15.00 h on sunny days. To avoid the effect of soil temperature on the CT, the data were taken when the infrared thermometer viewed no soil because of high leaf coverage areas (Jackson et al. 1981; Babar et al. 2006; Reynolds et al. 2012; Pask et al. 2012). Biomass (NDVI) was taken at GS55 and GS69 growth stage (Gutierrez-Rodriguez et al. 2004; Pask et al. 2012). Chlorophyll content (SPAD) and canopy temperature (°C) were taken at GS55 growth stages. The Zadoks Decimal Code (Z) was used to describe plant growth stages of cereals (Zadoks et al. 1974).

#### **Statistical analysis**

To evaluate significant differences between genotypes, the analysis of variance was performed. The differences between genotype means of parameters were tested by the L.S.D test (0.05). Letter groupings were generated by using a 5% level of significance. Data were analyzed statistically for analysis of variance in the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using the L.S.D test (Kalaycı 2005). The regression equations were calculated according to Finlay and Wilkinson (1963) and Eberhart and Russell (1966). Regression graphs were used to predict the adaptability of genotypes and the correlations between the quality parameters were determined by Pearson's correlation analysis.

#### **Results and Discussion**

There were significant differences among genotypes and between years because of the fluctuation of the rainfall across two growing cycles (Table 1). The results of the study showed that yield and other parameters investigated in wheat genotypes varied depending on genotypes and environmental conditions (Table 2). According to the results, the average yield of the experiment was 6799 kg ha<sup>-1</sup>, but the highest yield was obtained with 7471 kg ha<sup>-1</sup> in Selimiye and 7077 kg ha<sup>-1</sup> in Bereket cultivars. The lowest yielding cultivar was Kate A-1. Biomass and canopy temperature was measured at the heading stage and there were significant differences among genotypes. An earlier study showed that there was a positive correlation between yield and biomass (Reynolds et al. 1994; Gutierrez-Rodriguez et al. 2004; Marti et al. 2007). In the study, there was a positive association between biomass and grain yield. It was measured that the mean biomass was 0.74, the highest biomass in Tekirdağ cultivars with 0.79 and the lowest biomass was determined in Kate A-1 cultivar.

The chlorophyll content of the genotypes was measured at the heading stage by using a SPAD meter. There was a high variation in chlorophyll content of the genotypes and the lowest was found 44.50 in cultivar Saban and the highest was 50.35 in cultivar Bereket and 50.00 in Selimiye. Chlorophyll content positively affected grain yield in the genotypes. Canopy temperature is generally related to yield under drought stress condition in wheat (Reynolds et al. 1994; Trethowan and Reynolds, 2007; Reynolds et al. 2001). The mean canopy temperature was 19.39°C. Minimum and maximum canopy temperature ranged between 18.06°C and 20.13°C among genotypes and the lowest canopy temperature was measured in genotypes G19-2012, Bereket, Selimiye and Aldane (Table 3).



The adaptation strategies of the plants to drought stress include drought escape, drought avoidance and drought tolerance. Among these strategies, escaping drought involves the completion of the life cycle before the onset of the drought period. Therefore, early maturity has been known as a major drought escaping mechanism (Chaves et al. 2002). Due to the fluctuation of rainfall mid-early genotypes generally are favorable in bread wheat in the Trakya region. Medium maturity is the preferred feature in the region. Days of heading ranged from 105.0 to 116.1 among genotypes and the mean value was 109.9 days. Cultivars Tekirdağ and Saban were early (days to heading) genotypes (Table 3). Plant breeders have tried to select and release intermediate varieties (Richards et al. 2001; Calderini et al. 1999). Plant height and stem structure is a quite important trait in a wheat breeding program for lodging resistance. Table 3 shows that the mean plant height was 101.3 cm, the lowest and highest plant height ranged between 78 cm and 112 cm under rainfed conditions. The shortest plant height was scaled in cultivars Tekirdağ and followed by Saban and G11-2012 genotypes (Table 3).

The overall moisture-deficit-induced reduction in yield was primarily due to the reduction in kernel weight; the effects of moisture deficit on the yield of specific cultivars were largely due to the effects on kernels per spike. Cultivars x moisture treatment interactions were highly significant for test weight and yield. The effect of moisture deficit on kernel weight also was reflected in reduced test weight (Guttieri et al. 2001). Thousand kernel weight and test weight in wheat varied by genotypes and genotypes x year interactions (Table 2, 3 and 4). In the study, 1000-kernels weights (TKW) were very variable among genotypes and ranged between 37.9 g (G19-2012) and 47.3 g (Aldane). Aldane and Pehlivan had the highest TKW and the mean TKW were 42.8 g. The test weight of the genotypes ranged between 75.9 kg and 80.0 kg, while the mean test weight was 78.08 kg. The highest test weight was obtained from Selimiye, which is followed, by Pehlivan and Aldane cultivar (Table 3).

#### **Correlation analysis**

Correlation coefficients were determined by Pearson's correlation analysis. In the study, some relations between investigated characters were examined and correlation coefficients among the tested characters of cultivars were given in Table 4. Higher biomass positively affected grain yield and a moderate positive correlation were observed between grain yield and biomass (r=0.333). It was found a moderate positive correlation between grain yield

and thousand kernels weight (r=0.205). There was a negative correlation between plant height and grain yield so genotypes which have shorter plant height gave more yields (Table 4). According to results, there was a negative correlation between biomass with chlorophyll content (r=-0.241), days of heading (r=-0.512), plant height  $(r=-0.738^{**})$ , 1000-kernels weight (r = -0.357) and test weight ( $r = -0.682^*$ ). These resulted indicated that higher biomass significantly caused to decrease in the test weight and 1000-kernel weight of the genotypes. Low canopy temperature was scaled genotypes that have late heading and higher plant height. So, a negative correlation was determined between canopy temperature and days to heading (r=-0.554) and plant height (r=-0.171)(Table 4). These results showed that to see the expected relationship between grain yield with physiological and agronomical traits, physiological researches should be carried out under both various drought stress and rainfed environment conditions.

Grain yield is affected by environmental fluctuations and there are various components, such as some physiological traits, morphological and agronomic traits related to grain yield. In this study, some of the characters given above were examined and assessed for the relationship amongst them and were presented in Figure 1. There was a negative relationship between biomass and test weight ( $R^2=0.464$ ), plant height ( $R^2=0.544$ ). Grain yields positively associated with biomass ( $R^2=0.110$ ) and grain yield was also negatively associated with plant height ( $R^2=0.311$ ). This result showed that short genotypes had higher yield potential. Similar findings were also reported by (Kant et al. 2011). A negative relation was found between canopy temperature and chlorophyll content

 $(R^2=0.816)$ . There was also a positive relationship between chlorophyll content and grain yield ( $R^2=0.263$ ) (Figure 1). These results indicated that an increase in biomass and chlorophyll content of the genotypes led to an increase in grain yield. Also, lower canopy temperature was scaled in late heading and tall genotypes.

#### Conclusions

Bread wheat production in the Trakya region is important because of the high yielding capacity in wheat thanks to favorable environmental conditions. However, the fluctuations in rainfall in April and May causes yield losses and low-quality products in wheat. Therefore, physiological and agronomical studies on wheat under various environmental conditions are needed. In this experiment, there were significant differences among genotypes. Higher biomass and chlorophyll content positively affected grain yield and also higher thousand kernels weight increased grain yield. Genotypes, which have shorter plant height, had higher yields. The higher biomass negatively affected and decreased chlorophyll content, thousand kernels weight and test weight. Higher canopy temperature negatively affected grain yield and chlorophyll content in genotypes. The lower canopy temperature was scaled genotypes which has tall plant and late heading. All these results showed that to get the expected relationship among grain yield and physiological characters, researches should be carried out under various drought stress conditions. Also, physiological parameters such as biomass, canopy temperature and chlorophyll content could be used for yield components under rainfed conditions. The higher canopy temperature significantly reduced grain yield and chlorophyll content of genotypes.

Months	Rainfall (mm)		Temperature (°C) 2012-2013			Temperature (°C) 2013-2014		
	2012-2013	2013-2014	Min	Max	Mean	Min	Max	Mean
October	46.1	30.7	5.7	34.0	18.9	-1.6	26.8	12.8
November	12.4	73.9	-0.9	24.0	12.2	-2.4	23.4	11.0
December	165.8	2.3	-6.2	17.6	3.6	-5.6	12.1	2.7
January	134.6	74.9	-7.7	18.2	4.2	-4.2	17.3	5.5
February	104.5	3.8	-0.7	18.8	6.8	-4.4	20.2	7.6
March	62.9	124.5	-1.7	23.6	9.8	-1.4	23.7	10.1
April	51.0	36.8	4.0	32.0	14.5	-0.1	25.5	13.6
May	11.0	61.7	4.9	32.9	20.8	4.0	32.1	18.6
June	26.6	68.8	11.4	36.2	23.3	10.3	33.6	22.9
Total/Mean	614.9	477.4	-7.7	36.2	12.7	-5.6	33.6	11.6

Table 1. The rainfall and temperatures for two growing cycles in Edirne location.

Table 2. Combined analysis of variance for bread wheat genotypes across two years for investigated parameters.

Parameters	Year (Y)		Genoty	pes (G)	$\mathbf{Y} \times \mathbf{G}$		
	MS	F Value	MS	F Value	MS	F Value	
GY	802550.0	102.66**	15737.5	4.87**	21167.8	6.56**	
TW	98.28	114.14**	14.34	19.91**	1.68	2.34*	
TKW	391.36	216.89**	70.49	106.41**	18.76	28.31**	
SPAD	177.56	66.00**	36.43	18.93**	5.83	3.83**	
NDVI	0.031	32.77**	0.0056	26.83**	0.0006	2.92**	
СТ	5.11	4.06ns	4.58	5.86**	0.98	1.26ns	
РН	1581.01	61.49**	492.71	28.85**	24.78	1.45**	
DH	4860.41	2448.76**	88.05	38.12**	65.05	28.17**	

\*, \*\* Indicate significances, ns: non-significant at P<0.05 and P<0.01, respectively

MS: Mean square, GY: grain yield (kg ha<sup>-1</sup>), TW: Test weight (kg), TKW: Thousand kernels weight (g), SPAD: Chlorophyll content, NDVI: Biomass, CT: Canopy temperature (°C), PH: Plant height (cm), DH: Days of heading (day)



No	Genotypes	GY	NDVI	SPAD	СТ	DH	РН	TKW	ТW
1	Kate A-1	5658c	0.70g	46.26c	20.03a	113.0b	116.4a	40.6d	78.4c
2	Pehlivan	6558b	0.72f	44.54d	20.05a	110.9cd	109.0b	46.8a	79.4ab
3	Gelibolu	6688b	0.74de	45.74cd	20.04a	108.1e	99.3d	42.2c	78.9bc
4	Tekirdağ	6875b	0.79a	45.16cd	19.66ab	105.0g	89.6f	40.3d	77.3d
5	Aldane	6967ab	0.73f	48.55b	18.96bc	110.8cd	103.9c	47.3a	79.4ab
6	Selimiye	7471a	0.72f	50.00a	18.83bcd	109.8d	98.8d	45.1b	80.0a
7	Bereket	7077ab	0.73f	50.35a	18.23cd	116.1a	107.9bc	42.3c	78.1cd
8	Saban	6792b	0.75cd	44.50d	20.13a	106.0fg	94.4e	44.5b	77.5d
9	G11-2012	6877b	0.73ef	46.05c	19.88a	106.6ef	94.4e	40.2d	78.1cd
10	G16-2012	6855b	0.76bc	46.45c	19.43ab	112.0bc	96.3de	44.5b	76.0e
11	G19-2012	6951ab	0.77b	48.98ab	18.06d	111.5bc	104.6c	37.9e	75.9e
Mear	1	6797	0.74	46.96	19.39	109.97	101.3	42.88	78.08
LSD	(0.05)	56.8	0.01	1.38	0.88	1.52	4.13	0.81	0.84

Table 3. Mean performance of genotypes based on yield, physiological and quality parameters across two environments conditions.

GY: grain yield (kg ha<sup>-1</sup>), NDVI: Biomass, SPAD: Chlorophyll content, CT: Canopy temperature (°C), DH: Days of heading (day), PH: Plant height (cm), TKW: Thousand kernels weight (g), TW: Test weight (kg)

Table 4.	Coefficient of	of correlation	among the	tested p	arameters of	the genotypes.
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Traits	GY	NDVI	SPAD	СТ	DH	РН	TKW
NDVI	0.333						
SPAD	0.513	-0.241					
CT	-0.559	-0.082	-0.904**				
DH	-0.137	-0.512	0.612*	-0.554			
РН	-0.558	-0.738**	0.273	-0.171	0.781**		
TKW	0.205	-0.357	-0.048	0.180	0.089	0.052	
TW	0.041	-0.682*	0.107	0.210	0.002	0.269	0.560

Significance at \* P<0.01, \*\* P<0.05, ns: not significant

GY: grain yield, NDVI: Biomass, SPAD: Chlorophyll content, CT: Canopy temperature (°C), DH: Days of heading (day), PH: Plant height, TKW: 1000-kernels weight (g), TW: Test weight (kg)









Plant height and biomass (NDVI)



Chlorophyll content and canopy temperature



Plant height and yield



Grain yield and chlorophyll content



Biomass (NDVI) and grain yield



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## Registration of "Yüksel" Barley (Hordeum vulgare L.) Variety

Yüksel is two rowed barley (*Hordeum vulgare* L.) variety developed by Transitional Zone Agricultural Research Institute (TZARI) and registered in 2020. Yüksel crossing is Sonata/CWB117-5-9-5//CW117-77-9-7 and YEA5252-0E-0E-0E-2E-0E pedigrees. The crossing was made in 1999 and the yield test began in 2005-2006 growing year.

Similar to İnce 04 and Sabribey, Yüksel is two rowed cultivar and its spike is moderately long. Depending on the growing conditions, plant height varies between 80 and 100 cm. Since Yüksel is medium early and has high adaptation ability, it can be grown in all Medium Anatolian Region and Transitional Zone of Turkey. Thus, high yield can be obtained from both high fertility and poor fertility soil conditions. Yüksel is tolerant to net blotch (*Pyrenophora teres*) and scald (*Rhynchosporium commune* (formerly known as *R. secalis*) diseases.

When appropriate environmental conditions and optimum agronomic practices are provided, yield potential is high. The highest grain yields were obtained from Gözlü location with 5561 kg ha<sup>-1</sup> and Yenikent location with 5980 kg ha<sup>-1</sup> in 2018-2019 growing season. Average yield of the variety in Eskişehir conditions was 4840 kg ha<sup>-1</sup>. Recommended planting rate is between 400-450 seeds/m<sup>2</sup>. According to quality test results, test weight 61.4-66.9 kg, thousand kernel weight 35.6-40.9 g, protein content 12.7-14.6%, under sieve value 15.0-1.8%.

Pre-basic and basic seeds of the Yüksel cultivar have been produced by Transitional Zone Agricultural Research Institute (TZARI). Certified seed of the Yüksel are produced by state farms.

Figure 1. Grain and field appearance of Yüksel barley variety. (Original)



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### Registration of "Ocak" Barley (Hordeum vulgare L.) Variety

Ocak is two rowed barley (*Hordeum vulgare* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2020. Ocak cross is Slad//Ast/82 Mva 018-4/3/Rod/4/NS525 with TEA2275-0T-0T-3T-11T-3T-0T pedigree. Crossing was made in 2006 and grain yield test began from 2014-15 growing year.

Ocak cultivar is two rowed cultivar and its spike is moderately long and compact. It resembles with cultivar Bolayır. Ocak is a medium-tall cultivar, similar to Bolayır. Plant height is between 84 and 98 cm depending on the growing conditions. It is medium early in maturity and as it has good adaptation, it has been grown throughout Trakya-Marmara and transitional zone region of Turkey. It gives high yield both on fertile and less fertile soils. It has resistance to winterkilling and is tolerant to medium drought conditions. Ocak is highly tolerant to powdery mildew (*Blumeria graminis* f. sp. *hordei*), net blotch (*Pyrenophora teres*) and scald (caused *Rhynchosporium commune* (formerly *Rhynchosporium secalis*). Yield potential is high however, high yield can be obtained if environmental conditions are favorable and accompanied with good agronomic practices. The highest grain yield obtained was 8506 kg ha<sup>-1</sup> in Edirne location in 2018-19 growing years. Mean yield of the variety testing experiment was 7152 kg ha<sup>-1</sup> in Trakya growing conditions. Suggested planting rate is between 450-500 seeds/m<sup>2</sup>.

Grain feeding quality is good. The mean values of some qualities of the variety testing experiment (2017 and 2019) are; test weight 67.2-71.0 kg, thousand kernel weight 35.6-41.1 g, protein content 11.2-12.8%, and sieve value 64.9-88.7%. The highest quality values during 2017 and 2019 growing seasons before of the variety testing experiments were; 1000-kernel weight50.5 g, test weight 73.8 kg, protein content 12.0% and sieve value 95.8%.

Pre-Basic and Basic seeds of the Ocak cultivar have been produced by Trakya Agricultural Research Institute (TARI). Certified seed of the Ocakare produced by both private companies and state farms.

Figure 1. Spike and grain of the Ocak barley variety. (Original)



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## Registration of "Eylül" Bread Wheat (Triticum aestivum L.) Variety

Eylül is winter bread wheat (*Triticum aestivum* L.) variety developed by Trakya Agricultural Research Institute (TARI) and registered in 2020. Anafarta cross is Sau41/Sad1/5/Agri''S''/093-44/3/ Kkk/ltd/Lov29/4/ FKong15//Bow/Pwn/6/1518-4-38K with TE5857-2T-1T-3T-1T-7T-0T pedigree. Crossing was made in 2002 and grain yield test began in 2010-11 growing year.

The spike of the Eylül cultivar is moderately long, white, smooth, with awn and compact. It resembles with cultivar Gelibolu, Köprü and Tekirdağ. The flag leaf is dark-green and with low glaucousity. Grain is oval, hard and red colour. Eylül is a medium-tall cultivar, similar to Köprü, Gelibolu and Tekirdağ. Plant height is between 85 and 90 cm depending on the growing conditions. It is medium early and as it has good adaptation ability; it has been grown throughout the Trakya-Marmara region and some other transitional zone parts of Turkey. It gives high yield both under high fertility and poor fertility soil conditions. It has resistance to winter killing and is tolerant to medium drought conditions. Eylül is tolerant to powdery mildew (Erysiphe graminis f. sp. tritici), stripe rust (Puccinia striiformis f. sp. tritici) and leaf rust (Puccinia triticina).

Yield potential is high however, high yield can be obtained if environmental conditions are favorable and

applied good agronomic practices. The highest grain yield obtained was 8821 kg ha<sup>-1</sup> in Tekirdağ location in 2017 and 2018 growing years. Mean yield of the variety testing experiment was 7217 kg ha<sup>-1</sup> in Trakya growing conditions. Suggested planting rate is between 450-500 seeds/m<sup>2</sup>.

Grain quality is good. The mean values of some bread-making qualities of the variety testing experiment (2017 and 2019) are; test weight 71.2-80.4 kg, thousand kernel weight 30.8-38.8 g, protein content 10.9-15.6%, absorption 54.4-58.2%, sedimentation (Zel) 40-64 ml, gluten index 82.7-100%, gluten value 27.9-34.9%, alveograph energy value (W) 167-260. The highest quality values in 2016-2017 growing seasons application of the variety testing experiment were; thousand kernel weight 36.4 g, test weight 78.7 kg, protein content 13.3%, gluten value 34.6%, gluten index 97.7% and sedimentation (Zel) 65 ml.

Pre-Basic and Basic seeds of the Eylül cultivar have been produced by Trakya Agricultural Research Institute (TARI). Certified seed of the Eylül are produced by both private companies and state farms.

Figure 1. Spike and grain of Eylül wheat variety. (Original)



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## Registration of "Irmak01" Pea (Pisum sativum L.) Variety

"Irmak01" is a pea (*Pisum sativum* L.) variety developed and registered in 2020 by Eastern Mediterranean Agricultural Research Institute (EMARI) of Turkey. The variety is well adopted to winter conditions of Mediterranean, Aegean and South East Anatolia Region of Turkey. "Selection Breeding Method" was used to develop the variety from single plant selected from local population source materials.

Plants of "Irmak01" variety are well adopted to mechanised harvest due to 70-127 cm plant height and

21-53 cm first pod height. Time to flowering is 50-90 days and time to physiological maturity is 102-138 days. Grain is light-beige colored and round shaped which has 16.8-20.3 g 100-grain weight. Water absorption capacity is 0.19-0.20 ml/grain; water absorption index is 0.91-1.05%; swelling index is 2.13-2.31%; eight mm sieve value is 50.8-51.8%. Protein ratio is 24-25%. Time requirement for cooking is 40-46 minutes.

Average grain yield of "Irmak01" variety in field tests is 2,2 t/ha.

Figure 1. Flower, grain and pod of Irmak01 pea variety. (Original)







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## Registration of "Topçu" Dry Bean (Phaseolus vulgaris L.) Variety

"Topçu" dry bean variety was developed by Transitional Zone Agricultural Institute and registered in 2016. Eskişehir-855 dry bean variety, one of the parents of Topçu is in dwarf growth form and horoz grain type. Although, it is a very good variety in terms of quality criteria, it has been removed from seed production programs due to its sensitivity to bacterial halo blight (Pseudomonas syringae pv. syringae) disease. In order to transfer the resistance genes against bacterial halo blight disease (*Pseudomonas syringae* pv. syringae) to Eskişehir-855 variety, it was hybridized with lines 4F-2834 and 4F-2832. In addition, in the continuation of the studies, in order to increase the tolerance of Eskişehir-855 variety against both bacterial and viral diseases, crosses were made with lines 4F-2834 and 4F-2805. As a result of these two crosses, Eskişehir 855/4F-2834-39//4F-2832/3/4F-2832 pedigree line and Eskişehir-855/4F-2834-39//4F-2805/3/4F-2805 pedigree line which were obtained using single plant selection method was hybridized in 2003.

Then, a single plant selection was made at stage  $F_5$  in 2008 and this material was taken to yield trials in 2011.

Topçu dry bean variety is dwarf (39-58 cm) in growth form and the first and only dermason (rowbean) type dry bean variety in our country. Having a dwarf growth form makes it suitable for harvesting with machinery and facilitates cultivated in large fields. The number of days of flowering varies between 39-71 days and the number of physiological ripening days between 107-124 days.

Although, its grain size is smaller than the other bean varieties of dwarf type, its yield capacity is higher than the others. The average yield in the registration trials is 200.2 kg da<sup>-1</sup>. Topçu is recommended for dry bean cultivating areas.

It has been observed that Topçu has a medium tolerance to leaf halo blight and root rot diseases. 100 seed number, hydration capacity, swelling index, cooking time and protein rate of Topçu are between 25-33 g, 0.29-0.32 g/grain, 2.05-2.17%, 39-46 min. and 22.3-22.7%, respectively and its cooking flavor is quite good. Breeder material and gradual seed production are conducted by Transitional Zone Agricultural Research Institute (TZARI).

Figure 1. Field appearance, pod and grain of Topçu dry bean variety. (Original)



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## Registration of "Yektay" Safflower (Carthamus tinctorious L.) Variety

YEKTAY is safflower (*Carthamus tinctorious* L.) variety developed by Transitional Zone Agricultural Research Institute (TZARI) and registered in 2019.

Safflower has a great potential for arid areas and can take place cropping pattern and rotated with wheat, barley and some legumes. It is a broadleaf, highly branched and annual industrial crop having different ways of use in the World. Safflower seed oil consists of nearly 90% unsaturated fatty acids like oleic and linoleic acids. Safflower oil has taken place for human nutrition as a raw material as well as for production of biodiesel in Turkey. Beside of these, safflower petal also uses for medical purposes, coloring food and raw material for cosmetic.

YEKTAY is similar variety to Balci in terms of morphologic characters. YEKTAY is spiny variety and its flower is pure yellow. Its plant height is about 80-100 cm and 1000 seed weight is 40-50 g. Seed oil content of this variety is 35-37%. In dry condition, seed and oil yield are 2000-2500 kg ha<sup>-1</sup> and 850-1000 kg ha<sup>-1</sup>, respectively. YEKTAY is superior in terms of oil yield.

Pre-basic and basic seeds of the YEKTAY cultivar have been produced by Transitional Zone Agricultural Research Institute (TZARI).

Figure 1. Head, grain and field appearance of Yektay safflower variety. (Original)



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# Registration of "Kocamaninci" Rice (Oryza sativa L.) Variety

"Kocamaninci" is a *japonica* type semi-dwarf rice (Oryza sativa L.) variety developed by Black Sea Agricultural Research Institute. It was approved to release in April 2020. The variety was derived from cross breeding method and their parental lines are Kizilirmak  $\times$  KA474. Crossing was made in 2007, initiated as a F<sub>1.3</sub> bulk selection until 2010, sustained as a  $F_{4.7}$  pedigree selection of a single panicle row between 2011 and 2014. Preliminary yield trial, yield trial, regional advanced yield trials conducted from 2015 to 2017. Variety registration yield trials conducted in 5 regions such as Samsun, Bafra, Edirne, İpsala, Gönen from 2018 to 2019. The variety had an 18% yield advantage over the standard cultivars such as Osmancik-97, Kiziltan, Edirne and Cammeo (Anonymous 2020). The variety has a moderate resistance to both leaf and neck blast, which are caused by the fungal pathogen Magnaporthe oryzae.

The variety is a short statured conventional rice cultivar highly resistant to lodging. The variety has an upright plant sight, additionally vertical panicle and leaf aspect. It has averaged 80-85 cm plant height in yield trials across Black Sea region as well as in Thrace and Marmara regions (Figure 1). The average flowering days were 83-85 days and maturity days were 130-135 days (Unan et al. 2020). The variety has demonstrated good yield potential in head-to-head comparisons with the standard checks. The average grain yield potential is 8-10 t ha<sup>-1</sup>. Average milling rate were 65%. The head rice milling rate of the variety was 5% higher than other varieties (Anonymous 2018).

The variety had featured that length, width, length/ width ratio and kernel 1000 seed weight of milled whole kernels as 6.1-6.2 mm, 2.8-2.9 mm, 2.1-2.2 and 22-23 g, respectively. Milled rice has an ideal transparency structure.

Turkish Plant Variety Protection has been applied for the variety. Breeder and foundation seed of the variety will be produced and maintained by Black Sea Agricultural Research Institute, 55300, Tekkekoy, Samsun, Turkey. Limited quantities of seed are available on request to the corresponding author for research purposes.

In conclusion, the variety has excellent features such as semi-dwarf plant length, resistant lodging, good yield potential and especially stable maximum milling rate.

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Figure 1. Field appearance, grain and milled whole rice of Kocamaninci rice variety. (Original)



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#### Journal article:

Toker C (1998). Adaptation of kabuli chickpeas (*Cicer arietinum* L.) to the low and high lands in the West Mediterranean region of Turkey. Turk J Field Crop 3:10-15.

Toker C and Canci H (2003). Selection of chickpea (*Cicer arietinum* L.) genotypes for resistance to ascochyta blight [*Ascochyta rabie*i (Pass.) Labr.], yield and yield criteria. Turk J Agric For27: 277-283.

Toker C, Canci H and Ceylan FO (2006). Estimation of outcrossing rate in chickpea (*Cicer arietinum* L.) sown in autumn. Euphytica 151: 201-205.

# Article by Digital Object Identifier (DOI) number:

Yasar M, Ceylan FO, Ikten C and Toker C (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (*Cicer arietinum* L.). Euphyticadoi:10.1007/s00109000086

#### Book:

Toker C (2014). Yemeklik Baklagiller. BISAB, Ankara.

# Book chapter:

Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM (2007). Abiotic stresses. In: Chickpea Breeding and Management, Yadav SS, Redden B, Chen W and Sharma B (eds.), CAB Int. Wallingford, pp: 474-496.

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FAOSTAT J (2013) http://faostat.fao.org/site/567/default.aspx# anchor. Accessed 15 May 2013.

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Yasar M (2012). Penetrance and expressivity of double podding characteristic in chickpea (*Cicer arietinum* L.). Dissertation, Akdeniz University, Antalya.

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# Abbreviations

Abbreviations should be defined at first mention and used consistently.







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