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Full Length Research Paper

Prospects of clonal selection for enhancing productivity in Saffron (*Crocus sativus* L.)

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Saffron a member of Iridaceae family is a perennial spice species. It is derived from the stigma of the flower of the saffron crocus (*Crocus sativus* L.), which is collected and dried to produce the spice. The main compounds that accumulated throughout stigma development in *C. sativus* L. were crocetin, its glucoside derivatives and picrocrocin, all of which increased as stigmas reached a fully developed stage. The volatile composition of *C. sativus* stigmas changed notably as stigmas developed with each developmental stage being characterized by a different volatile combination. In red stigmas, b-cyclocitral, the 7,8 cleavage product of b-carotene, was highly produced, suggesting the implication of both b-carotene and zeaxanthin in crocetin formation breeding of saffron (*C. sativus* L.), its position, urgency and topicality of the problem are considered in the present review. Clonal selection is proposed for genetic improvement of saffron in order to increase yield production and quality.

Key words: Saffron, clonal selection, variability.

INTRODUCTION

Saffron (*Crocus sativus* L.) belongs to the family Irridaceae and genus *Crocus*, of which about 80 spp. are so far known. Its cultivation in the world extends through 0 to 90°E longitude (Spain to Kashmir) and 30 to 45°N latitude (Persia to England). It is well known that saffron (*C. sativus* L.) is a very valuable irreplaceable spice with exceptional medicinal properties which has been cultivated since ancient times. Ancient history of saffron cultivation goes back to many thousands years ago. It was originated very likely in Greece and then distributed in the other Mediterranean and Near East countries (Turkey, Italy, Azerbaijan, Iran, Iraq, North of India, etc.). There are no wild forms and it exists solely in culture. Being triploid with chromosome number 2*n*=3*x*=24 and basic number of *x*=8, saffron never bears seed and it is propagated exclusively in a vegetative way by corms. Its vegetation period starts in autumn, continues through winter and finishes in the middle of spring. Therefore its growth and development is very slow, facing the cold period of the year which results in low productivity of the plant. All over the world saffron is known as one cultivar, as descent of certain triploid sterile plant arisen once spontaneously in nature which was caught by sight of man and involved into cultivation (Mathew, 1977). Saffron, the dried red stigmas of *C. sativus*, has been used as flavouring and colouring agent since then and is currently considered the world's most expensive spice. Saffron is made up of a complex mixture of volatile and non-volatile compounds that contribute to its overall aroma and flavor (Moraga et al., 2009)

Propagation

Cytological, DNA, and reproductive studies on the allied species of *C. sativus* such as *C. cartwrightianus*, *C.*

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thomasii, *C. hadriaticus*, indicate a more likely parent of saffron may be *C. Cartwrightianus*or and *C. thomasii*. Both species are diploid with a karyotype similar to saffron. In addition, their pollen can fertilize the egg cell of saffron, giving rise to seeds which are viable, germinate and form new corms. Thus saffron can originate through fertilization of a normal reduced egg cell with an unreduced male gamete of the same *Crocus* species or by crossing between an egg cell and the male unreduced gamete of another species. The saffron is a sterile geophyte that produces annual renewal corms and is propagated only by them (Mathew, 1982). The physical properties of saffron corms are prerequisite to designing and developing harvesting, handling, sizing and sowing equipments of corms (Hassan-Beygy et al., 2010).

Cytological studies indicate that saffron is a sterile triploid (2n=3x=24) plant. The origin of saffron by allopolyploidy seems more probable considering the recent data on its karyotype and molecular biology. Many authors have hypothesized the origin of saffron by autotriploid (Mathew, 1977). On the basis of overall length and centromeric position, the somatic chromosomes could be assembled in seven triplets, one pair and one single chromosome. The karyotype consists of a series of chromosome triplets with the exception of one group of three chromosomes of which one is different from the others. Because of this unique chromosome in saffron genotype, selfing can be excluded (Chichiricco, 1984). Since *C. sativus* is sterile, the presence of this chromosome may be explained by the existence of chromosomal polymorphism at diploid level in the progenitors, most probably *C. Thomasii* or *C. cartwrightianus.*

Saffron has been propagated and still continues to be propagated vegetatively. There is a supposition that saffron as a clone can be scarcely changed genetically and its improvement is hardly possible through clonal selection (Dhar et al., 1988, Piqueras et al., 1999). Meanwhile, other suppositions exist as well. For example, Rzakuliyev (1959) investigating in Apsheron (Baku) specimens of saffron obtained from 6 regions (2 regions in Italy, France, Istanbul, Yalta and Mashtağa) during 3 vegetations in 1934 to 1937 concluded that it is possible to create a new high yielding cultivars of this plant on the basis of clonal selection. Kapinos (1965) studied the morphogenesis and cytoembryology of *C. sativus* under climatic conditions of Apsheron and came to the conclusion that this plant represents variegated blend of genetically heterogeneous forms – clones, and clonal selection on it would be very promising. Apparently, the lack of new cultivars of saffron at present may not be explained by the impossibilities of the improvement of this plant through clonal selection. To solve this problem, researchers need to try clonal selection for achieving improvement in saffron. Many researchers have addressed the problem of clonal selection of saffron, including a large group from Azerbaijan who have tackled this problem forthright (Rzakuliyev, 1959; Kapinos , 1965; Agayev 1993; Agayev, 1994b; Agayev et al. 2007). In 1981, Dhar et al. (1988) surveyed the natural populations of Kashmir saffron (*C. sativus* L.) recording the range of variation and tabulating perianth length, perianth width, length of the colored part of the stigma, flower number/stigma, fresh weight/stigma and dry weight/stigma. These authors together with Piqueras et al. (1999), however, assumed that saffron as a clone cannot be altered genetically to any major extent and that its improvement is hardly possible through clonal selection. In Kashmir, Munshi and Zargar (1991) identified an elite subpopulation developed from the progenies of corms selected from extensive saffron belts. Munshi (1992) derived information on coefficients of variation, heritability and genetic advance using data on ten yield components in 11 diverse saffron genotypes (mostly from Jammu and Kashmir with some exotic varieties) grown between 1986 and 1989. Significant genotypic differences were observed for all characteristics, except day to 100% flowering. The coefficients of variation were greatest for the number of daughter corms/mother corm and number of flower/ space. The highest yield for dry saffron was obtained in 1987 by SKUAST (Sher-e-Kashmir University of Agricultural Sciences Technology of Kashmir) genotypes Nag c8708 and Bodi c8606 (3.57 and 3.30 kg/ha, respectively). GrilliCaiola et al. (2001) reported phenotypic differences in terms of flower size, tepal shape and color intensity. Pistil weight was found to show a wide range, revealing the possibility of saffron improvement through selection. Similar results of wide ranges of variability have also been reported by Zargar (2002) in Kashmir. The productivity, growth and quality attributes of ten saffron accessions of Birjand, Ghaen, Gonabad, Torbat-Haydarieh, Ferdows, Istahban, Kerman, Isfahan and Shahr-Kord were studied under natural environmental conditions at ShahrKord in Central Iran. Ehsanzadeh et al. (2004) concluded that the three latter accessions could be grown when satisfactory stigma yield is the goal. Picci (1987), however, found only small differences in yields from saffron grown in Abruzzi, Emilia and Sardinia. Indian experts, in accordance with the results of Iranian authors in similar studies, have confirmed the yield superiority (Table 1 and Figure 2) of ten genotypes (e.g. SMD-3, SMD-11, SMD-31, SMD-45, with 4.3, 4.2, 4.8, 7.6 kg/ha of dry pistil yield, respectively) (Nehvi et al., 2007a, b). A comparison of many hundreds and thousands of clones, each grown from one corm of the same weight, resulted in the identification of ‘‘superior’’ clones in terms of exceptionally large numbers of flowers and large (>10 g) corms. Based on the number of flowers and number of large corms, which are the two most economically important attributes of saffron, the clones were classified as extraordinary, superior, ordinary, inferior and declining clones. This background information illustrates that the

Table 1. Performance of elite saffron clones available with SKUAST-K (Gowhar et al., 2011).

Genotype	Saffron yield (kg/ha)	No. of daughter corms	Average corm weight (g)	Crocin content (%)
SMD-3	6.30	6.26	5.37	15.49
SMD-11	6.20	4.79	5.40	13.88
SMD-31	6.8	6.32	5.66	14.81
SMD-45	7.6	5.06	8.20	17.10
SMD-52	5.4	4.55	7.63	13.89
SMD-68	5.3	3.45	8.44	16.63
SMD-79	5.4	2.91	8.49	16.91
SMD-81	5.2	3.20	7.49	16.59
SMD-211	6.06	4.12	5.18	15.57
SMD-224	5.5	3.04	4.25	14.92

and importance of clonal selection with reference to improving the traits of cultivated saffron thus suggesting that in the existing plantations clonal selection of saffron is possible and promising.

Having an ancient history of cultivation, saffron apparently may contain a lot of genetically changed forms (clones) as the result of spontaneous mutations in somatic cells. The task is to find and study the individually, to separate the economically valuable forms and to bring them to the new industrial cultivars. Thus clonal selection of saffron can help us to isolate the genetically diverse superior clones which can be used as cultivars with higher number of flowers and higher quantity of large corms.

HOW NEW CLONES OF SAFFRON DEVELOPED

Clone of saffron is the vegetative progeny of supposed initial triploid plant and population of saffron is mixture of different clones. During evolution, populations of saffron have been cultivated and propagated, and various mutant clones have appeared. These mutant clones never shared their gene pool with other plants. Each mutant clone evolved and multiplied independently. Today, the populations of saffron represent a conglomerate of very different clones that have arisen as a result of numerous changes during cultivation by man. Despite their different vitality potential, these clones do not compete, as they are cultivated by the man under the same identical conditions. The application of clonal selection in saffron means that clones with (many) excellent parameters should be selected for, even when they have certain undesirable characters. It is not possible to eliminate the identified defects due to the sterility of saffron. Therefore, it is essential that those clones designated for selection should necessarily have all positive attributes. Extraordinary and superior plants of saffron significant selection value can be separated from the general population of plants, tested again and then transformed, by propagation, into exclusively high-yielding cultivars. Two main difficulties of saffron breeding through clonal selection are as follow.

Recognition of new clones

In any plantation, saffron is represented by plants existing in highly different “ages” of individuals connected with different sizes of corms underground. Above the ground, these plants differ in the size and number of their flowers (at the stage of flowering), also in their number and size of leaves. If some plants are sharply different from the others in certain characters, for the aim of breeding, a researcher cannot practically identify them. Naturally he does not see corms underground, and cannot elucidate the cause of the differences whether these differences are due to the age (size) of corms, or because of the genotypes of plants. So, genetically different (if existing) and similar plants will continue to grow together and not be subjected to selection.

Multiplication of clones

Let us suppose that farmer recognizes certain plant(s) which could be used as a clone with good economic characters. Multiplication of such clone(s) would be a problem. One saffron corm with proper care produces an average of 4 corms of middle size during vegetative growth (one year). At such intensity of propagation, it could be brought to about 1 million corms after 17 years. This amount could be enough for planting on the area of 2 ha. It is clear that a farmer will never accomplish such an exhausting work of many years. Therefore the farmers would not pursue the aim to make new cultivar of saffron even if they have been lucky to find some clones with very highly expressed economically valuable characters. Concerning the researcher, in our opinion, selection of potential clone can be multiplied for development of new cultivar. Alternatively, superior selections of saffron could be propagated rapidly via *in vitro* technique (Homes et al.,

1987). Investigations in this direction are very promising. Unfortunately the experiments pursuing rapid corm propagation of saffron have not been successful so far and a few *in vitro* developed corms had been produced. Matured corms of *in vitro* origin in mass production had not been produced. Accordingly *in vitro* propagation protocols need to be refined and strengthened.

METHODS OF CLONAL SELECTION OF SAFFRON

With an objective of breeding new cultivars of saffron with economically valuable traits, two approaches need to be followed;

1. Selection of superior clones in existing plantations,
2. Creating new valuable forms through induced mutations (experimentally).

Selection of superior clones in existing plantations

Corms of saffron are selected from the different regions where the crop has been cultivated from ancient times and corms collected from different collection areas are kept separately. The peel is removed from all selected saffron corms and corms are classified into groups based on weight. The corms are divided in groups on weight groups, that is 3.0 to 3.9, 4.0 to 4.9, 5.0 to 5.9, 6.0 to 6.9, 7.0 to 7.9, 8.0 to 8.9, 9.0 to 9.9, 10.0 to 10.9, 11.0 to 11.9, 12.0 to 12.9 g, and so on. The corms of each weight group are planted separately in rows in the field at different places in pits; the corms were planted in rows separately in the field with one corm per hill (PIT). The distance between adjacent rows is 50 cm and between pits is 50 cm. The plants of each identified pit are inspected during flowering at 3rd and 4th generation after planting. The pits are labeled, indicating the number of flowers and dates (Agayev et al., 2009).

The traits noted are:

1. Pits with highest no of flowers.
2. Pits with ordinary number of flowers.
3. Pits with minimal (one to three) number of flowers
4. Pits with complete absence of flowers.

After four years of vegetative generation and complete study, all of the corms from all labeled pits are dug out and packed in proper packages of each pit separately. Each package is marked with the following data:

1. Name of the region
2. Number of flowers (registered at flowering time in 4th vegetative generation)
3. Any other data if noted

The corms of each package is weighed and divided into three groups

1. Corms weighing >10 (large corms)
2. Corms weighing <10 but > 5 (middle corms)
3. Corms weighing <5 (small corms)

These clones in packages will be objects of further study at successive stages of selection with the target of achieving higher number of flower and quantity of large corms (Agayev et al., 2009). Through clonal selection, superior plants of saffron can be identified and selected from among a large number of plants selected from different areas. The selected plants will undergo continuous study and selection for many generations and at the end the selected plants will be assessed for yield with the aim of being released for commercial use. So clonal selection will result in creation of new cultivars of saffron with substantial increase in productivity.

New concepts in clonal selection

1. Big corm index: Percentage of the weight of big ‘corm (>10 g) from the total weight of all corms of the given clone.
2. Multiplication index: Designates how many times the weight of the planted initial corm has increased during four vegetative generations; ‘
3. Flower creating index: Average corm weight, which was necessary for formation of one flower at a given generation of the clone. ‘‘Corm set’’.
4. Cormset: Indicates the complete set of corms (weight, number and size) dug out from one pit after a certain generation. The ‘‘Corm set formula’’ is developed to show the relationship between the numbers of large, middle-sized and small corms; for example, 8, 3, 7 indicates that there were eight, three and seven large, middle-sized and small corms, respectively.

RESULTS OF SAFFRON SELECTIONS IN KASHMİR

A survey of fifteen saffron growing villages of Kashmir by SKUAST-K has lead to the identification of ten prominent elite clones through clonal selection with distinct superiority in terms of yield and quality over natural populations .Three conjugate pairs of primers viz., SA-C+SA-T, SA-D+SA-S and SA-E+SA-R produced a consistent banding pattern of 0.5 to 1.0 size in 2% Agarose gel. Perusal of Table 1 revealed that the total number of bands obtained from the conjugate primer pairs from 10 genotypes ranged from 1 to 7 (Table 2). The maximum number of scorable bands (that is, 7) was obtained from SA-D+ SA-S primer pair, whereas, the primer pair SA-C+SA-T and primer pair SA-E+SA-R showed 1 and 2 scorable bands, respectively. The only primer pair, that is, SA-D+SA-S showed maximum number of polymorphic bands (that is, 7). Molecular characterization of ten elite saffron genotypes based on 21 random amplified polymorphic DNA (RAPD) markers revealed a considerable amount of genetic diversity among tested genotypes (Figure 1). Similarity index based on Jaccards coefficient ranged from 0.375 to 0.834 and the maximum similarity coefficient (0.834) was observed between the genotype SMD-45 and SMD-79. The dendrogram based on molecular data divided the tested genotypes in two clusters. However, genotypes including, SD-3, SMD-45, SMD-79 and SMD-68 formed the cluster I and genotypes including, SMD-11, SMD-52, SMD-81, SMD-211, SMD-224 and control formed cluster II at similarity coefficient of 44%, which showed a high level of genetic diversity between two clusters containing different genotypes. A different level of variability was observed among different genotypes within each cluster also. The primer pair, that is, SA-D+SA-S was found to be the best primer pair which showed maximum number of scorable and polymorphic bands (Nehvi et al., 2007a;

Table 2. Number of scorable and polymorphic RAPD bands obtained by PCR amplification of DNA of *Crocus sativus* L.

Primers	Scorable bands	Polymorphic bands	Polymorphism (%)
SA-C + SA-T	1	0	0
SA-C + SA-T	7	7	100
SA-C + SA-T	2	0	0
Total	10	7	70

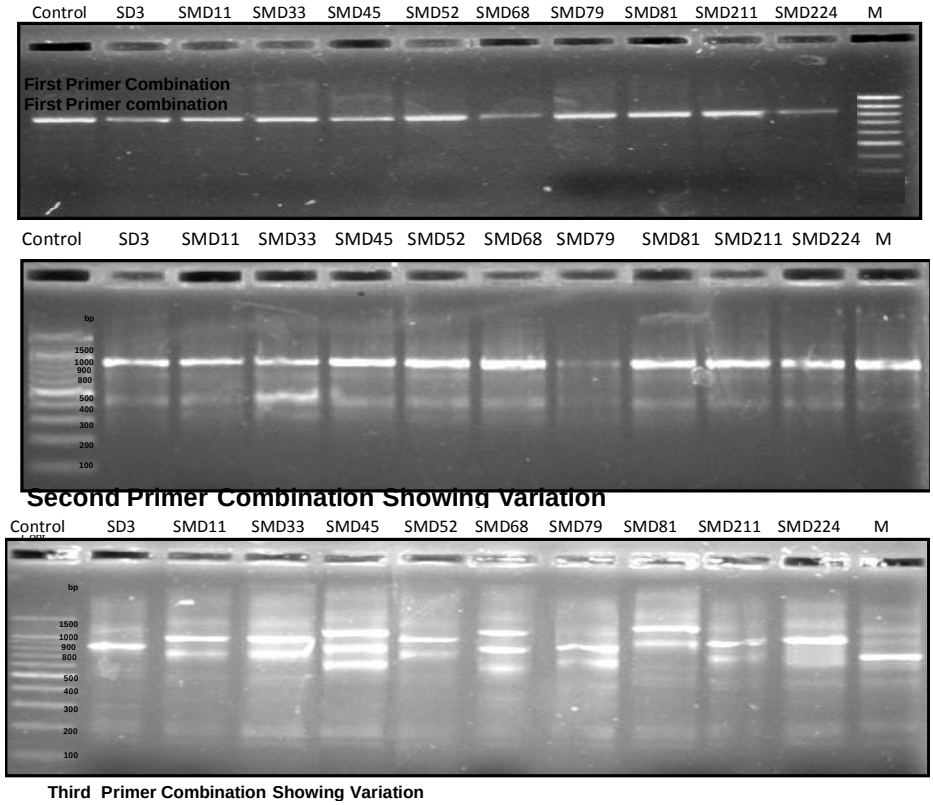

Figure 1. Variability at molecular level (Imran et al., 2010).

Imran et al., 2010).

The present investigation was carried out during 2007 on ten elite saffron genotypes viz., SD-3, SMD-11, SMD-31, SMD-45, SMD-52, SMD-68, SMD-79, SM-81, SMD-211, SMD-224 and one sample of natural population being superior in saffron yield and quality. Genetic diversity was studied using polymerase chain reaction (PCR) based amplified polymorphic DNA (RAPD) markers as described by William et al. (1990).

A survey of prominent saffron (229 in number) growing areas of Kashmir comprising 15 villages (Zeevan, Khrew, Wuyan, Ladhoo in Srinagar district; Dusso, Namlabal, Konibal, Chandar, Pampore, Barsu, Lathipora in Pulwama district and Chadora, Chararisharief, Kakawring, Hapatnar in Budgam district) located at an altitude of 1686, 1644, 1597 and 1730 m.a.s.l. The remaining genotypes collected from Zeewan, Khrew, Wuyan, Ladoo, in district Srinagar, Dusso, Namlabal, respectively was carried out during August 2003 and 60 corms samples of uniform weight and size (>10.0 g/3.5 cm) were collected from each location. Data pooled over years revealed that cluster I accommodated 229 genotypes followed by cluster II (2) (Table 1). Konibal, ChadharPampore, Barsoo, Lethipora, Koil in district Pulwama and Chadora, Charieshariefl, Kakawring, Hapathanar, Nagm in district Budgam got grouped in cluster I except for genotype SDM-140, SDM-138 from Lethipora (cluster II) SDM-116 from Konibal (cluster III and XI), SDM-61 from Dassu (cluster IV and V); SDM-235 from Ladoo (cluster VI), SDM-45 from Zeewan (cluster VIII) and SDM-224 from Nagam (cluster IX). The pattern of group constellations indicated that the

Figure 2. Performance of elite genotypes available at Saffron Research Station, Pampore SKUAST-Kashmir, India.

geographical diversity was not an essential factor to group the genotypes from a particular source. This means that geographical diversity, though important, was not the factor in determining the genetic divergence. The highest intra-cluster distance was observed between genotype SDM-43 and SDM-220 grouped in cluster I. Cluster VIII and X accommodating genotype SDM-45 and SDM-115, recorded maximum inter-cluster distance (445.51) followed by cluster VI and X (416.74), cluster II and cluster XI (416.38) and cluster VIII and cluster XI (415.82). Cluster VIII revealed high cluster mean for saffron yield, average weight of daughter corms and pistil length on account of grouping of high yielding genotype SDM-45, showing saffron yields to the tune of 15.30 mg associated with 17.10% crocin content. Percent contribution of different traits towards divergence revealed strong influence of fresh pistil weight, stigma length and crocin content. Therefore, such characters can be taken as criteria in selection for divergent lines (Makhdoomi et al., 2010).

Creating new valuable forms induced mutations

Induced mutations play an important role in creating new variations in clonally propagated plants because mutations in such plants may easily get stabilized and can be manipulated. So mutation breeding offers a scope for induction of variability in saffron for its subsequent utilization (Agayev et al., 1975; Nehvi et al., 2010; Muzaferova, 1970). For successful establishment of any crop on commercial scale the availability of adapted cultivars with desired characters is prerequisite, therefore, an attempt was made to create variability for floral, morphological and anatomical traits in saffron using physical and chemical mutagens. Corms at different stages of growth rate were subjected to different doses of physical and chemical mutagens. The study revealed that Colchicine (mostly used as an anti mitotic agent, 0.05%) was found beneficial for enhancing pistil length, leaf number and leaf length with a negative effect on survival corm treatment with radiation dose of 0.2, EMS (0.1%) and Ethidium Bromide (0.2%) showed significant positive effect on survival, increased number of heavier flowers and pistil weight. Mutagens induced morphological and anatomical variants showing increased number of stomata, thicker and broader leaves (Figure 3). Floral variant induced in M1 through 0.2 KRad radiation was not a stable character. Resting bud stage (1^{st} -15^{th} June) has been identified as appropriate stage for inducing variation through physical and chemical mutagens. Plants showing phenotypic variations are being maintained for further observations

Conclusion

Our results suggest that saffron (*C. sativus* L.) populations are not homogeneous, despite their clonal origin. The application of clonal selection in saffron propagation means that clones with (many) excellent parameters should be selected for, even when these contain any serious defect (or defects). It is clearly not possible to eliminate the identified defects separately from

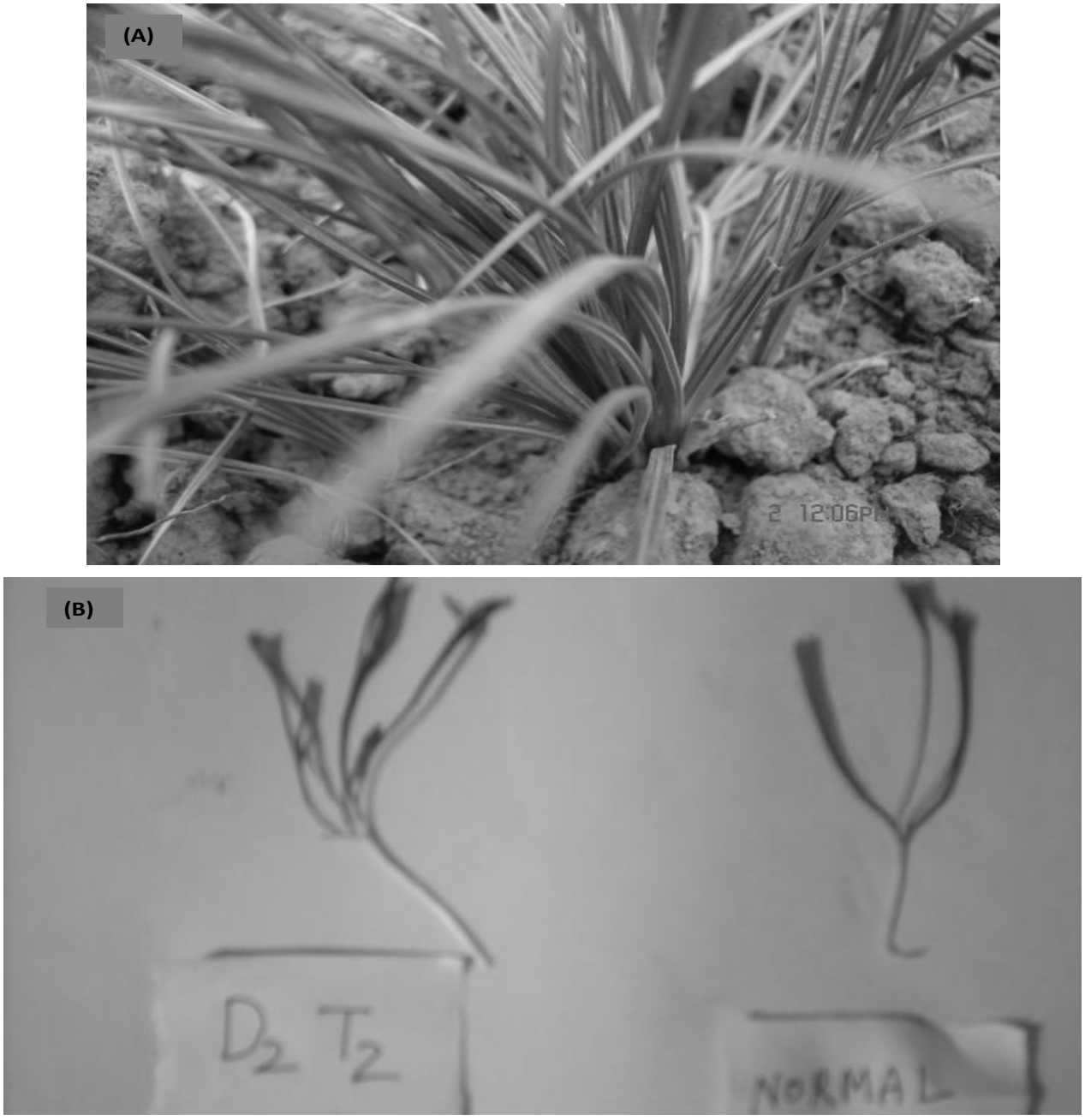

Figure 3. Morphological variants induced through (A) Gamma radiation 0.2 kr; (B) Ethidium Bromide 0.2%.

from the rather valuable other attributes due to the sterility sterility of saffron. Therefore, it is essential that those clones designated for selection should necessarily have the entire complex of positive attributes. In pursuit of creating new high-yielding and high quality cultivars of saffron, it is necessary to establish that the existing populations of saffron are rich in terms of genetic and selection potential. We have accomplished this by a method of simple clonal selection, revealing new and extraordinary and superior plants with a complex of positive attributes and creating new cultivars with high practical advantages. Each identified extraordinary or superior plant is potentially a new cultivar; it is only necessary to propagate it and introduce it in practice as a new cultivar.

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