MORPHOLOGICAL AND MOLECULAR GENETIC CHARATERIZATION OF HEARTSEASE (*VIOLA TRICOLOR*) CULTIVARS IN Egypt

By

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B.Sc.Agric.Sci. (Genetic), Fac.Agric.,Ain shams Univ.,2001

A Thesis Submitted in Partial Fulfillment

Of

The Requirement for the Degree of

MASTER OF SCIENCE
in
Agricultural Sciences
(Genetics)

Department of Genetics

Faculty of Agriculture

Ain Shams University

2017

Approval Sheet

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ABSTRACT

Heba Mansour Megahed Ahmed: Morphological and Molecular Genetic Charaterization of Heartsease (*Viola Tricolor*) Cultivars in Egypt. Unpublished Ph.D., Thesis, Department of Genetic, Faculty of Agriculture, Ain Shams University, 2017.

This study was carried out through five successive years (2010-2015). The aim of this work was to select plants according to the survival plants in the farm, survival of the flowers on the plant, the flower diameter, shape and color of the flower (*V.tricolor*, *var.canina*, *var.biflora*, *var.alpina* and *var.calcarate*).The use of three different doses irradiation of gamma rays (10- 20- 40 Gy) for 2min, given the new genotype coming from different new radiation through morphological. Detect a biochemical DNA analysis. Finally evaluate some of the new genotypes.

Genotype (*V.tricolor* var.biflora) was recorded new genotype with radiation dose of gamma rays (10 Gy) different in colour of the flower, appearance whitish by four petals made up in white and one yellow petal.

Genotype *(V.tricolor* var.calcarate*) were* recorded new genotype with radiation dose of gamma rays (10 Gy) different in colour of the flower, appearance whitish by more where the flower of five petals. The genotype *(V.tricolor* var.calcarate) were recorded with radiation dose of gamma rays (20 Gy) appearance of the flowers is completely different in the colour of the flowers, gave a new category entirely different in shape and the highest percentage of survival the flowers on the plant, number of internodes main branch, plant length, flower diameter, flower number and leaf number on the plant while dose of gamma rays (40 Gy) the lowest result.

Number of bands that resulted was a 18 band with the initiator OP-C05 and 13 bands when using the initiator OP-C04 and the number 20 band in the event of the use of the initiator OP-C01 the molecular weights between 112 to 1250 base pair.

ACKNOWLEDGEMENT

First and for most, I wish to express my deep thanks for "ALLAH" who gave me the ability to finish my study.

I wish to express my sincere thanks, deepest gratitude and appreciation to **prof. Dr. Ashraf Bakry Abd El Razik**, Professor of Genetic, Department of Genetics, Fac. of Agric., Ain Shams Univ. for his supervision valuable advice, constructive criticism and continuous guidance.

I am grateful to **prof. Dr .Samir Abdel-Aziz Ibrahim,** Professor Emeritus of Genetics, Department of Genetics, Fac. of Agric., Ain Shams Univ. for guidance, discussion and reviewing the manuscript.

Special thanks and deepest gratitude to **Dr. Ayman Kamal Ibrahim,** Lecture of Ornamental Plants, Department of Horticulture, Fac. of Agric., Ain Shams Univ. for their valuable advices and guidance.

I am indebted to the late **Dr. Ahmed Othman Hamada**, Senior Research, Dep., of Fruit and Ornamental Plant Breeding. Horticulture Research Institute, Agriculture Research Center.

I am deeply grateful to **Dr. Nahla Awad**, Senior Research, Dep., of fruit Breeding and Ornamental Plant Horticulture research Institute, Agricultural Research Center (A.R.C), for providing the facilities to carry-out this work.

Many thanks to all my staff in Dep., of Fruit Breeding and Ornamental Plant Horticulture research Institute, Agricultural Research Center (A.R.C), specially thanks to **Dr. Eman Hamed** and **Dr. Yasser Samair** for their sincere help, additive support and guidance.

I would like to thank all my lovely family especially to **my father**, **my mother**, **my husband** and **my children** to support me my preoccupation bathing in search of work.

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INTRODUCTION

Flower textures have very high ornamental of landscape and are an important breeding target. Since flower texture can also improve ornamental value and increase the diversity of flower colour, they are a very important characteristic in creating new flower color variants.

Viola genus contains about 500 species, (**Qaiser and Omer, 1985**) widely, spread all over the world and about 30 species are growing wild in Romania. In Pakistan it is represented of 17 species by genus viola and are distributed in various localities (**Perveen and Qaiser, 2009**).

The modern pansy hybrid between *Viola ×Wittrockiana Gams* is thought to have derived from *Viola tricolor*; it is among the species abundantly present on heaps in the Olkusz area **(Hildebrandt *et al.*, 2006**). A native of central Europe, a perennial in cooler climates that exhibits reduced flowering under warm temperatures **(Schwabe, 1985).**

It is small plant of creeping and ramping habit and can hoist itself. It grows in short grassland on farms from September to April or late-spring. It is propagating by seed (**Bartok, 1992**). High temperatures can inhibit flower induction initiation, and/or development (**Abdul-Baki, 1991**). In the past 50 year's new pansy colors such as shades of pink, rose, and orange had become available. Modern pansy breeding is largely concentrated in Germany, United States, and Japan.

It is have single blooms. Each with five petals that are rounded in shape. (**Qaiser and Omar, 1985**). There is a wide color range for pansy flowers is red, purple, blue, bronze, pink, yellow, white, lavender, and orange.

INTRODUCRUIN

There are three main categories of pansies based on flower size; Large / 3.5 to 4.5 inch-diameter blooms, Medium / 2.5 to 3.5 inch-diameter blooms and Multiflora / 1.5 to 2.5 inch-diameter blooms (**Carlson, 1990**).

V. tricolor can be divided into pure-color flowers and multicolored flowers. Pure-colour varieties have a single colour on the flower and are called "clear". Multicolored flowers that have a very dark blue/black centers are called "blotched "or faced some blotched pansies may have a different color blotch. Other multicolored white or light colored edges or have petals of differing colors; most of these two or three color also have a dark face **(Carlson, 1990 and King, 1992)**.

Economically, *V.* tricolor is ranked the third value in the total sales in the United States behind *Pelargonium* and *Petunia hybrida*, in total sales value in 2004 **(USDA-NASS, 2005),** with a wholesale value of 151 million dollars in USA (**Niu *et al*., 2000**).

The aims of the present investigation are

1- Assesment of pansy genotype cultivated in Egypt and selection best genotypes.
2- Determine the effect of different doses of gamma rays on four genotypes of *V.tricolor*.
3- Introduce new genotype have the following criteria
   a- The length of survival of the plant.
   b- Extend the longevity of flowers survival on the plant.
   c- Introduce new colour of flowers with new genetic combinations.

REVIEW OF LITERATURE

1- Annuals ornamental plants

Structure of flowers is significant ornamental characteristics. Reflected light from the exterior of petals, inclusive colorful bracts and leaves, is a key factor defines the structure of flowers. The results of experience in which we examined diverse species of flower petals refers to that there are two types of reflected light: one is superficial reflected light, which is defines by the form of the epidermal cells. More light is reflected by flat epidermal cells than by papillate ones. In certain, when the angle of incident light was varied, the belt-shaped reflected light was only spotted through the microscope from the side of the papillate epidermal cells. The other type of reflected light is dispersed reflected light, which is defines by the petal structure, and the higher the pigment content in a petal, the more light is soak up. The intensity and origin of dispersed reflected light depends on the volume and site of air spaces in the petal that are dividing among the epidermal, palisade, and spongy cells **(Zhang *et al*., 2008).**

Genus viola (Violaceae) consists of approximately 500 species widely distributed throughout the world. In Pakistan, seventeen (17) different species of viola are abundantly found. Traditional healers have been patronizing various species of this genus in numerous disorders since time immemorial. Some of them are already validated scientifically such as antifungal antibacterial, antiplasmodial, antihypertensive, antidyslipidemia, anticancer, analgesic, antipyretic, anti-inflammatory, diuretic, anthelmintic, antioxidant, anticancer **(Naveed *et al.*, 2012*)***.

Viola genus contains about 500 species; widely spread all over the world and about 30 species are growing wild in Romania. While *Viola tricolor L*. (wild pansy) is considered the most important, there are known some other indigenous species: *V.arvensis Murray*, *V. odarata L*., *V.*

declinata Waldst. Intensive breeding programs have performed for unique flower colors, large flower size, greater flower number, and temperature tolerance have led to many new and exciting cultivars to select from for use in the landscape **(Anca *et al*., 2010**). New species of viola (Violaceae) from north-western Yunnan, China, is substantive and described. Viola *dimorphophylla* Y. S. Chen & Q. E. Yang sp. nov. Is endemic to Zhongdian County, north-western Yunnan, and is very easily distinguishable from all other Chinese species of the genus that characterized dimorphic leaves, with the basal ones being long petiolate, undivided and widely cordate **(You Sheng and Yang, 2005).**

2- Characterization of *Viola tricolor*

V. tricolor have single blooms, each with five petals that are rounded in shape. There is a wide color range for pansy flowers. Colors include red, purple, blue, bronze, pink, black, yellow, white, lavender, orange, apricot, and mahogany. Pansies can be divided into pure-color flowers and multicolored flowers. Pure-color varieties have a single color on the flower and are called 'clear.' Multicolored flowers that have a very dark blue/black centers are called 'blotched' or 'faced.' Some blotched pansies may have a different color blotch than the usual dark blue/black face. Other multicolored pansies have white or light colored edges or have petals of differing colors; most of these two or three color pansies also have a dark face **(Polking *et al*., 1990*)***.

In the last 50 years, new pansy colors such as shades of pink, rose, and orange have become available. Modern pansy breeding is largely concentrated in Germany, the United States, and Japan. From the late 1970 up to today, pansy breeding has concentrated on aspects of quality such as vigor, heat tolerance, and free flowering **(Bartok, 1992*)***.

New species of Viola L., *Viola yildirimlii*. From South Anatolia is substantive and described by **(Dinc and Yidrml, 2003).** It is found on the

rocky slopes of Aladag National Park, in the county of Adana, south Turkey, at a rising of 1800 m. It belongs to Viola, subsect, and is identical to the Turkish endemics *Viola isaurica Contandr*. Diagnostic morphologic advantage for a detailed discrimination from two identical taxa and other Turkish eflagellatae species are discussed. Pollen morphology of Violaceae, a family of c. 825-900 species **(Mark *et al.*, 2012).**

3- Important of viola

Wild pansy contains (leaves) 0.3% 0f salicylic acid and its derivative such as the methyl ester and viol utoside; phenol carboxylic acids; 10.0% of mucilages; 2.4-4.5 % of tannins, flavonoids; carotenoids; coumarins; umbelliferone; small amounts of saponins; ascorbic acid and tocopherol **(Bisset and Wichtl., 2001 and Savickiene *et al.*, 2002).**

Wild pansy is used externally and internally. The therapeutic activity of pansy has been identified in treating various skin conditions, such as eczema, seborrhea, impetigo, acne, catarrh of the respiratory tract, and whooping cough. The herb is employed in treating frequent and painful urination in conditions such as cystitis. The salicylates contained in the plant are anti-inflammatory. Due to the high concentration of rutin, in the herb, it may be employed to prevent bruising and broken capillaries, to check the buildup of fluid in the tissues and to help to reduce blood pressure. The drug is mildly laxative. It was formerly in much repute as a remedy for epilepsy and numerous other complaints, and the flowers were considered as cordial and helpful in treating diseases of the heart, from which may have arisen its popular name of Heartsease as much as from belief in it as a love potion. *Viola tricolor L*, was formerly official in the US pharmacopoeia, and is still employed in America in the form of an ointment and poultice in eczema and other skin troubles, and internally for bronchitis. The over ground part of wild pansy has been

employed for their mucilaginous, demulcent and expectorant properties. In the older folk medicine the drug of pansy was considered to be a 'blood-cleansing' agent, *i.e.* it was supposed to deploy a metabolism-promoting action; it was employed as an adjuvant for appropriate indications, as diuretic, diaphoretic, and as well as in rheumatism, arthritis, and arteriosclerosis **(Bakšyté *et al*., 1973 ; Bisset and Wichtl., 2001).** It can gently alter the functioning of nerves, and the immune system. It is helpful in cases of nightmares, insomnia, and distressed sleep with frequent night awakenings. The herb of wild pansy may be very successfully used after surgery to prevent reoccurring tumors **(McGuffin *et al*., 1997).**

The cyclotides are disulfide-rich plant proteins that are exceptional in their cyclic structure; their N and C termini are joined by a peptide bond, forming a continuous circular backbone, which is reinforced by three interlocked disulfide bonds. Cyclotides have been found mainly in the coffee (Rubiaceae) and violet (Violaceae) plant families. Within the Violaceae, cyclotides seem to be widely distributed, but the cyclotide complements of the vast majority of Violaceae species have not yet been explored **(Buiman *et al*., 2010)**.

Cyclotides are small disulfide-rich peptides that are characterized by a head-to-tail cyclized peptide backbone and a knotted arrangement of three conserved disulfide bonds. They are present in many plants from the Violaceae, Rubiaceae and Cucurbitaceae families, with individual plants expressing a suite of dozens of cyclotides. So far >140 sequences and 15 three-dimensional structures have been determined but it is estimated that the family probably comprises many thousands of members. Their primary function in plants is thought to be as defense agents, based on their potent insecticidal activity, but they also have a range of other biological activities, including anti-HIV, antimicrobial and cytotoxic activities. Because of their exceptional stability they have attracted interest as templates for protein engineering and drug design applications.