Journal of Horticultural Science
Vol. 30, No. 3, Fall 2016, P. 121
ISSN: 2008 - 4730

نشریه علوم باغبانی (علوم و صنایع کشاورزی)
جلد 30، شماره 3، پاییز 1395، ص. 121
شاپا: 2008 - 4730

Determining Cytological Developments of Microspore in Four Varieties of Tomato (*Lycopersicum esculentum* Mill)

R. Najib[1*], M. Farsi[2]- A. Mirshamsi kakhki[3]- S.R.Vessal[4]

Received: 07-07-2014
Accepted: 05-10--2015

Introduction: Homozygous doubled haploid lines production through induction of androgenesis is a promising method to accelerate the classical breeding program. However, this technology is relatively under-developed in tomato so that improvements in methodology are required. Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetables which in addition of it is importance as a food, is utilized as a model plant for cytological and cytogenetic studies. Tomato breeding programs are often based on the production and selection of hybrid plants. To produce hybrid plants and application of features that is needed to breed pure lines with high specific combining abilities, new technologies such as doubled haploid production through induction of androgenesis can be an effective strategy to provide pure lines in tomato. One of the critical factors for induction of androgenesis in tomato is to use of microspores being in appropriate developmental stage. Cytological examination is one of the most accurate methods for determining the correct stage of microspore development. In this study, a number of characteristics were evaluated including the cytological properties of normal microspores development and pollen grains as well as the relationship between length of flower bud and anther length.

Materials and Methods: In this study, four varieties of tomato including Mobil-Netherlands, Baker, U. S. Agriseed and Khoram were chosen. To determine the appropriate stage of microspore development for Anther culture, cytologycal studies were accomplished at different size length of flower buds (2.0-7.9 mm). Collection of flower buds to conduct experiments was done during 10-40 days after flowering for each cultivar. Flower buds collected early in the morning hours and within the containers closed-door ice were transported to the laboratory. To investigate the correlation between the length of flower bud and anther length, randomly selected from within each group of three flower buds, and their length was measurement. Then anthers were removed and anther length was measured for each flower buds. A total of 240 anthers, sixty anthers from each cultivar, were examined by microscope. In order to examine the development stage of microspores and pollen grains, flower buds at different length (5-10 mm) were calculated. Flower buds were incubated at 4 ^{o}C for 15 minutes and stained in acetocarmin %4 solution and squashed. In order to determine the relative frequency of each stage of the development of microspore and pollen, microspores at least 100 randomly in different parts of prepared slides were counted. Average relative frequency of different stages, meiosis, tetrads, microspores young and old and young and mature pollen grains with a standard deviation was calculated. Cytological studies were accomplished by microscopy research Olympus B X 51 and photographed by a digital camera D P 70. All analysis was conducted using statistical software JMP 8.

Results and Discussion: The time of anthers collection for the induction of haploid is very crucial. In order to determine the appropriate steps to carry out pre-treatment induced changes in the normal development of microspores embryogenesis and cytological properties in various stages of division and development should be monitored. The results showed that there was a significant correlation between the length of flower bud and the anther length ($r = 0.8$, $P < 0.0001$). Cytological studies showed that the normal pathway of microspore development in tomato could be divided into three phases: meiosis to tetrad formation, tetrad separation, differentiation and maturation of microspores. At each stage, microspore size and morphological characteristics were different. The highest frequency of meiotic microspores stage to the mid-uninucleate stage was in length 4.0-4.9 mm of buds.

Conclusions: According to the results of this study can be replaced flower buds during the anther criteria to determine the appropriate microspores cultured in vitro to be used as a significant correlation between the length of the flower buds and anthers over there. The findings can be used to determine the appropriate steps for

1, 2 and 3-MSC. Student, Professor and Assistance Professor Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad
(*-Corresponding Author Email: Najib.r.1367@gmail.com)
4- Assistance Professor Department of Research Legumes, Research Center For Plant Sciences, Ferdowsi University of Mashhad

pretreatment, changes in the normal development and implantation of embryos used microspore. It seems that it could be possible to determine the right time to harvest flower buds for deviation of normal development of microspores to saprophytic pathway to induct haploid callus or embryogenesis.

Keywords: Androgenesis Induction, Haploid, Microspore Development