Ulud. Üniv. Zir. Fak. Derg., (1997) 13: 155-163

Somaclonal Variation and Factors Affecting Somaclonal Variation

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ABSTRACT

Inefficient selection and screening procedure and lack of genetic variation in some varieties reveal necessity of finding new sources for selection of desirable variants in plant breeding. Although mutation techniques and wild species obtain from gene banks have been used for increasing of genetic variation, somaclonal variation (genetic variation induced by cell and tissue culture) offers a great opportunity to increase the genetic variations of crops. The occurrence of somaclonal variation has been stressed by numerous researchers and displayed in many crops[1,2]. Somaclonal variation is successfully applied for selection of agronomically important traits such as disease and stress resistant variants in plant breeding. Factors affecting somaclonal variation are described in this review.

Key words: Somaclonal variation, in vitro selection, tissue culture.

ÖZET

Somaklonal Varyasyon ve Somaklonal Varyasyona Etki Eden Faktörler

Bitki ıslahında, yetersiz seleksiyon ve seçme prosedürü ile genetik varyasyonun eksikliği bazı varyetelerde arzu edilen varyantların seçilmesi açısından yeni kaynakların bulunması gerekliliğini ortaya çıkarır. Mutasyon teknikleri ve gen bankalarından elde edilen yabani türler genetik varyasyon tabanının arttırılması için kullanılsa da somatik varyasyon (hücre ve doku

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kültürleri tarafından indükte edilen genetik varyasyon) ürünlerin genetik varyasyonunu arttırmada büyük olanak sağlar. Çok sayıda üründe hücre ve doku kültürleri tarafından genetik varyasyonun açığa çıktığı birçok araştırıcı tarafından rapor edilmiştir[1,2]. Somaklonal varyasyon bitki ıslahında agronomik açıdan önemli olan özelliklerin özellikle hastalıklara ve strese dayanıklı varyantların seçiminde başarılı olarak uygulanmaktadır. Bu makalede somatik varyasyona neden olan faktörler tanımlanmıştır.

Anahtar sözcükler: Somaklonal varyasyon, in vitro seleksiyon, doku kültürü.

INTRODUCTION

Using cell and tissue culture techniques reveals genetic variation in crop plants and their progeny. This is defined as somaclonal variation[3]. Many types of genetic changes occur in somaclonal variation including alterations in DNA sequence e.g. single gene mutation, transposition, amplification; in gross chromosome structure e.g. duplications, translocations, deletions; in chromosome number e.g. polyploidy or aneuploidy; and in chloroplast or mitochondrial genomes[4,5,6]. These types of changes are stable through succeeding generations. However, the variation exposed as a result of a tissue culture cycle can be non-heritable (epigenetic) which would not be transmitted through meiosis and it may be reversible during the life of a plant. Hence it is worthless for sexually propagated plant production. Changes have also been identified that are both heritable and unstable[5].

Somaclonal variation can be influenced by a combination of factors. These include; the species and genotype (the ploidy level), tissue culture procedures employed, time and frequency of subculture, the source of explant and the composition of the culture medium[1,7,8,9].

The somaclonal variation obtained from tissue, cell and organ culture technology and factors affecting somaclonal variation are briefly reviewed in this study.

1. THE SOURCE OF EXPLANT

It has been thought that the variation in plants regenerated from tissue culture was pre-existing in the cells of the donor explant, either as a somatic variation or residual heterozygosity[10]. Explant tissue may not be genetically homogeneous and heterogenecity may be magnified by the proliferation of differing cell types. Mainly, the influence of tissue source would be most stressed on polysomatic species. Generally speaking, plant cells differentiated from polysomatic plants may contain polyploid and aneuploid constitutions.

Thus mesophyll protoplasts of Su/su (heterozygous, light green) tobacco plant gave 2,156 calli, of which 79 produced plants. Of these 79 colonies, 25% were phenotypically homogeneous (Su/Su dark green, su/su pale) and the remaining 75% of colonies were heterogeneous. These findings point to either extremely early mutational events or to variation preexisting in the protoplast[11]. The effect of explant type on the variation of tomato cultures was found little difference in regenerated plants derived from different explants except from hypocotyls which produced 58 % polyploid cells[2]. Different frequencies of polyploidy and aneuploidy have been reported on pea *(Pisum sativum)* tissue cultures[12]. Callus obtained from leaf explants consisted of over 90 % diploid cells whereas in stem callus only 70% was diploid and in root callus only 50%. Potato plants were regenerated from leaf, stem, rachis and tuber explants. It was noticed that only tuber pieces were found to give higher levels of variation, with over 50% plants aneuploid in contrast with less than 10% from other explants[13]. *Solanum brevidens* plants regenerated from cotyledon explants were tetraploid at a frequency of 70%, while 20% were tetraploid in regeneration from leaf pieces[14].

2. THE COMPOSITION OF CULTURE MEDIUM

Mutagenic action of media components, especially hormones, has often been demonstrated. The influence of different hormones on the ploidy level of callus derived from hypocotyl segments of *Nigella sativa* were studied[15]. The synthetic auxins NAA (1-naphthaleneacetic acid), IBA (indole-3-butyric acid), IAA (indole acetic acid) caused steady decrease in the normal diploid cells over the time studied while 2,4-D (2,4- dichlorophenoxyacetic acid) resulted in a more rapid shift away from diploidy. It can be said that this is likely to be an indirect action related to promotion of rapid disorganized growth rather direct mutagenic properties of the auxins. Diploid suspension cultures of carrot which were grown for 90 weeks, 0.1 mg/l 2,4-D caused significantly higher frequency of multipolar anaphases and lagging chromosomes by spindle failure[16.17]. Later work showed that above 30 mg/l 2,4-D completely prevented spindle formation. The frequent establishment of fresh cultures, the use of suitable medium and subculture regimes can maintain clonal fidelity in both cultures and regenerated plants[18]. In sugarcane, regenerated somaclones resistant to sugarcane mosaic virus were obtained from a susceptible variety by increasing the number of subcultures of the embryogenic callus in MS medium supplemented with 3 mg/l of 2,4-D. DNA fingerprint results showed that resistant somaclones had different genetic constitutions from the maternal line[19]. There is not many evidence for a direct effect of media components on gene mutations. The frequency of 0.5 Per 100 strains resulting in a change from blue (heterozygous) to pink

(homozygous) in the *Tradescantia* stamen hair system was increased by 2,4-D. Spontaneous mutant events were revealed in this system[20].

3. THE SPECIES AND GENOTYPE

Somaclonal variation can be influenced by the genotype of the donor plants. Plants regenerated from two cultivars of oat, Lodi and Tippecanoe produced different frequencies of cytogenetically abnormal plants. 49% of Lodi regenerated plants and 12% of Tippecanoe regenerated plants were abnormal after 4 months in culture[6]. It was shown that the genotype of the donor had a significant effect on the extent of variation generated during culture. In soybean, the frequency of somaclonal variation in poplars of the *Leuce* section (8%) was higher than in those of the *Aigeiros* and *Tacamahaco* sections (1%). It was shown in this study that regenerated variants were tetraploid or heteroploid while original clones were all diploid[21]. The genetic structure of source plants that already show low or moderate levels of resistance can affect successful selection for disease resistance[8]. In celery, a much higher frequency of plants highly resistant to Fusarium yellows (*Fusarium oxysporum* f. sp. *apii*) was regenerated from embryogenic suspension cells of a moderately resistant cultivar than from highly susceptible source material[22]. Recently, similar results have been also found in potato by using callus cultures induced from stem explants of a cultivar (Désirée) tolerant to *Verticillium dahliae*. *Verticillium* culture filtrates were applied to single node cuttings for *in vitro* selection of resistant clones and then regenerants were infected with fungal conidia to confirm the resistance[23].

4. TIME AND FREQUENCY OF SUBCULTURE

There is evidence that the length of the culture period has a significant effect on the extent of variation generated during culture. Prolonged suspension cultures of carrot generated higher frequencies of tetraploidy, octoploidy and aneuploidy within the cells, but it was also associated with reduced embryogenic potential[24]. Long term maintenance of carrot callus cultures on medium containing 2,4-D also resulted in entirely aneuploid cells in callus. However, these callus cultures lost their ability to form embryos[25]. In oats (*Avena sativa* L.), it was noticed that the frequency of cytogenetically abnormal, regenerated plants increased dramatically with increased time in culture. Frequency of observable chromosome aberrations (trisomics, monosomics, interchanges and plants with deficient chromosomes) increased in one cultivar from 49% after 4 months of culture to 88% after 20 months. Some strains of *Pisum sativum*, after prolonged period of subculture, showed a wide range of chromosome numbers at higher ploidy levels but completely lacked diploidy[6]. The loss in root regeneration capacity was related to the increase in abnormality of chromosomal constitution[26]. Higher level of

resistance to sugarcane eyespot *(Helminthosporium sacchari)* toxin in regenerated plantlets was obtained with prolonged callus cultures[27]. The frequency of cytogenetically abnormal regenerated maize plants was increased with culture age. The age effect was not due to an increased mutation rate, but was due to mutational events that occurred throughout culture development with subsequent maintenance and accumulation of aberrant cells over time[28]. Morphogenic callus was diploid while non-morphogenic callus was found to contain high frequencies of aneuploidy, triploidy, tetraploidy and octoploidy in barley. As a result of increased chromosome numbers, regeneration acted as a barrier against the more extreme variants as a loss of organogenesis is related to a high degree of aneuploidy[29]. However, it was also demonstrated in potato that calluses exhibiting high levels of aneuploidy are still capable of shoot regeneration, giving wide ranges of chromosome numbers in regenerated plants[30]. Although embryos and plants were produced from long-term carrot cultures, these plants were either sterile or formed very few seeds which did not survive after germination[31]. Recently, somatic segregation as a part of genetic variation was shown in carrot hypocotyl explant. The meiosis-like divisions at 1-3% was observed in hypocotyl explants, in the presence of auxin[32]. Cytological investigations of carrot cell lines which were kept long term in culture revealed the ranges of chromosome numbers e.g. new levels of ploidy and novel chromosome numbers. Mainly aberrant divisions resulted in two haploid prophases and metaphases, appeared as a segregational process, during which the chromosome number is halved from 2n (diploid embryogenic cell line) to n (haploid cell line)[33].

The length of interval between subcultures may also be important in somaclonal variation. Short subculture intervals were found necessary for maintenance of chromosome stability in cell suspensions of *Nicotiana spp.* Suspension cultures subcultured to fresh medium at 7-day intervals showed a notable decline in the frequency of tetraploid cells within the diploid culture of carrot[34]. Linear growth and stationary phase periods of carrot suspension cultures were eliminated by 7-day subculture regime while maximum growth rate and mitotic index of cultures did not change[35,36].

5. TISSUE CULTURE PROCEDURE EMPLOYED

The tissue culture procedure employed can also affect variation. Meristems cultured without a state of dedifferentiation produced little or no variation in contrast to when a dedifferentiated state was induced[4,37]. Protoplast regenerants tend to be more variable than those produced directly from leaf or stem tissues. Carrot protoplasts (isolated from cell cultures) treated with polyethylene glycol (PEG) to induce protoplast fusion resulted in a higher frequency of tetraploid and hexaploid chromosomal structures in

regenerated plants (41.2%) than those grown from untreated protoplasts (16%) and from the original cells (6.6%)[38].

Cultured carrot cells exhibited substantial variation in chromosome number, both ploidy and aneuploidy and chromosome morphology, whereas regenerated plants were diploid, with the exception of a few tetraploids and they showed no cytological abnormalities[39].

USE OF SOMACLONAL VARIATION IN PLANT BREEDING

A number of methods are used to increase the variation at the cytological, molecular, cytoplasmic, and epigenetic levels. Somaclonal variation plays an important role to reveal new genetic variation in intact plants and offers great opportunity for selection of agriculturally useful variants at the cellular level. There are a number of advantages of somaclonal variation as described below 1. It is a cheap form of biotechnology compared with somatic hybridization and transformation 2. novel variants have been reported among somaclones 3. It is rapid and easily accessible source of variation used in plant breeding. Somaclonal variation and *in vitro* selection can be applied in many economically important crops in many aspects. Examples of beneficial changes have included male sterility in tomato, rice and maize, earliness in maize and sorghum, increased dry matter in potato, increased yield (without other changes) in oat, frost resistance in wheat, disease resistance in wheat, maize, rice, sugarcane, sugarbeet, potato, tomato, herbicide and insecticide resistance in alfalfa, tobacco, maize, salt and drought tolerance in tobacco, alfalfa, sugarbeet[2,7,9,40]. On the other hand there are also many disadvantages of it. The main drawback of this method is always not possible to recover useful variants. The variation may be in a negative direction, other aspects of the plants might be altered in a negative way and in positive changes, all the changes obtained may not be novel and stable[1]. Therefore it is necessary that a large number of lines must be screened for selection of desirable characteristic.

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