http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (8): 745-749, 2003
ISSN 1028-8880

Attempts of Haploidy Induction in Tomato (*Lycopersicon esculentum* Mill.) Via Gynogenesis I: Pollination with *Solanum sisymbriifolium* Lam. Pollen

[1]Ugur Bal and Kazim Abak
[1]Department of Horticulture, Faculty of Agriculture, Trakya University, Tekirdag, Turkey
Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Turkey

Abstract: Haploid induction potentials in *Lycopersicon esculentum* Mill. (2n=2x=24) via unilateral pollination with *Solanum sisymbriifolium* Lam. (2n=2x=24) pollen were investigated. In April, the cv. Invictus set 12 fruits out of 72 pollinations and, the remaining six cultivars did not set fruit. Whereas in May, from a total of 242 pollinations onto eight varieties, 26 fruits were obtained from only six of the varieties. In addition to the ovules obtained from the cv. Invictus fruits, cv. Sagit 146 fruits contained 65 ovules. While cv. Invictus ovules gave rise to plantlets, ovules from cv. Sagit 146 were lost due to contamination. Chromosome counting of eight in vitro plants of cv. Invictus showed cells with 24, 25 and 26 chromosomes. Haploid chromosome numbers were not observed in any of the cells studied. Potentials of the approach were also discussed.

Key words: Wide pollination, *in situ* pollen tube development, chromosomal instability

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the most produced vegetable crop in the World (Anonymous, 2002) and variety improvement has been continuous to meet the demands of various crop production and protection practices and needs (Kalloo, 1993). In general, production of new varieties is realized via hybrid breeding methods by which production of F1 hybrid varieties, displaying characteristics of higher performance than their parents, require pure or inbred lines which are produced either by repeated selfings if applicable, or doubling the chromosome number of the haploids.

Anther culture, the commonly practiced method for the haploidy in tomato, has not met breeders' satisfaction (Chlyah *et al*., 1990) and, alternative methods of haploid production have not been reported to date.

F1 varieties, containing desired characteristics introgressed via interspecific or intergeneric crosses, are mainly used in the tomato crop production (Kalloo and Banerjee, 1990; Kalloo, 1993; Stevens and Rick, 1994). For the production of F1 varieties *Lycopersicon peruvianum*, taxonomically most distant to tomato, *L. chilense*, *L. hirsutum*, *L. pimpinellifolium* and *Solanum lycopersicoides* have been used with varying degrees of success (Rick *et al*., 1987; Gradziel and Robinson, 1991; Lan Zhuang and Adachi, 1996; Chetelat *et al*., 1997; Doganlar *et al*., 1997; Vidavski *et al*., 1998; Egashira *et al*., 1999; Foolad and Lin, 2001).

Previous interspecific or –generic hybridization reports dealt only with gene introgression and perhaps overlooked its potential for the induction of haploids of parthenogenetic origin. In general, haploid production through the induction of a haploid cell of the embryo sac can be realized using interspecific or –generic crosses. In this way, either the haploid egg cell is induced to develop and produce the embryo (Hougas *et al*., 1964) or a complete chromosome set of one of the parents is eliminated from the developing diploid zygotic embryo (Kasha and Kao, 1970) resulting in haploids or doubled haploids.

Use of *Solanum sisymbriifolium* Lam., a member of the *Solanaceae* family, has been previously reported for gene introgression purposes in *Solanum melongena* (Bletsos *et al*., 1998). In addition, fruit set and plant development were determined when *Solanum sisymbriifolium* pollen was placed on the *Lycopersicon esculentum* stigmata, but the report lacked detailed information on pollen germination, pollen tube development *in situ* and the details of the progeny obtained especially regarding haploidy. Alternatively, however, some cells with the haploid number of 12, in addition to the diploid number of 24, were observed, but all the eight progeny developed into fertile plants (Chambonnet, 1996).

Therefore, bearing in mind the potentiality of haploid production of tomato via distant hybridization, the present work focused on unilateral intergeneric hybridization of *Lycopersicon esculentum* with *Solanum sisymbriifolium* Lam.

Materials and Methods

The experiments were conducted at the plastic greenhouses and laboratories of Department of

Horticulture, Faculty of Agriculture, Cukurova University, Turkey during March-June 2001.

Growing of plants: Seeds of only one genotype of *Solanum sisymbriifolium* were obtained from Dr. M.C. Daunay, INRA, Montfavet, France and sown in the previous year in seed trays. Developing seedlings were grown in the field and pollen was obtained from fully matured plants. Similarly, seeds of a total of eight tomato varieties, namely Falcon, Gokce, Elif, Sagit 146, Invictus, SC2121, Y-410-F1 and Y-18-90, were sown in seed trays and seedlings at the 3-4 true leaf stage were transplanted to plastic greenhouses with 0.4 m and 1.25 m in and between row distances, respectively.

Pollinations: Pollinations were carried out twice both in April and May. Flowers, one to two days prior to anthesis, were emasculated by removing petals and others. Stigmas, now exposed to open air, were not further protected before or after pollination. Several pollinations were carried out onto each tomato variety and the *S. sisymbriifolium* combination and two to three unpollinated flowers were left as the control.

Observation of *in situ* pollen tube development: In order to observe pollen germination on the stigmas, pollen tube development in the style and entry of tubes into ovules, 24 and 48 hours following pollination, at least three samples of each combination of pollination were removed and fixed in 3:1 glacial acetic and ethanol. Following a washing of 12 h under running tap water, the samples were softened in 8 N NaOH for 6-8 hours and washed once more for 12 hours, then transferred to anilyn blue dye. Samples left in the dye for at least 24 h in the dark were then examined microscopically under UV light. The stock of anilyn blue dye was prepared by mixing of 1 g of anilyin blue and 11.28 g tripotassium phosphate in 250 ml of distilled water. A working solution was prepared by diluting one part of the stock solution in three parts of distilled water.

Rescue and culture of ovules: To overcome the risk of embryo abortion, fruits were harvested early in development. Ovules containing embryos were cultured following dissection for which fruits were removed 24 and 36 days after pollination and, immediately after, sterilized in 10% NaOCl solution containing 2-3 drops of Tween 80. Following sterilization, the fruits were washed three times in distilled water and cut open. Ovules observed were dissected out and cultured in jars containing basic MS medium (Murashige and Skoog, 1962) without growth regulators.

Chromosome counting from *in vitro* growing root tips: Tips of actively growing root tips, ca. one cm in length, were removed from the *in vitro* plantlets of 5-10 cm length. The root tips were collected at 10-11 o'clock in the morning and following a brief washing in tap water, transferred to 8-hydroxyquinoline solution for mitotic arrest at 4°C for 24 hours, then fixed overnight in a mixture of 3:1 ethanol and glacial acetic acid at room temperature. Before staining, the root tips were hydrolized in 1 N HCl at 60 °C for 15-20 minutes. The hydrolyzed root tips were stained in Feulgen for at least 2 hours in the dark and magenta coloured, i.e. ca. 2 mm long tips, were macerated on slides in a drop of acetocarmine making temporary slides.

Results and Discussion

Crosses were carried out twice, both in April and May 2001 and in the earlier pollination only 12 fruit sets (32.4%) were obtained from the cv. Invictus, being the only cultivar, among the seven, resulting in fruit set, out of a total of 237 pollinations (Table 1). In May pollinations, using two more cultivars, a total of 242 flowers were pollinated and fruit set was obtained in five of the eight cultivars (Table 2). While the highest fruit set was 25% from the cultivar Y-18-90, the lowest was 6.8 % from the cv. Elif. Regarding the cv. Invictus, the rate of fruit set decreased from 32.4% in April to 17.9% in May.

To confirm *S. sisymbriifolium* pollen tube growth and its entry into ovules, in situ pollen tube development was researched in flowers from May pollinations. Pollen tubes developed in situ through the style and entered the ovules within 24 hour of the pollination in all the cultivars except for the cultivars Y-410-F1 and Y-18-90 in which pollen tubes developed only to the halfway of the style. Pollen tube development in 48 hour samples was similar to the 24 hour samples in all the cultivars tested (Table 2; Fig. 1). Although pollen tube entry in the ovules was readily visible in six of the eight varieties, it is uncertain whether the fertilization took place. In the cv. Falcon, despite the observation of the pollen tube entry into the ovule, fruit set was not observed.

A total of 12 fruits obtained were from the cv. Invictus in the April pollinations and 10 of the fruits were cut open to rescue the embryos before a probable abortion. Of the 12 fruits obtained from the cv. Invictus in the April pollinations, 10 were cut open and seven of the fruits were empty without developing ovules. From the remaining three fruits a total of 14 ovules were dissected out and cultured. Ovules cultured were of three types, i.e. 10 were light green and larger then all, three medium and one small. Four large ovules and one medium, germinated approximately two weeks after the initial culture. Although

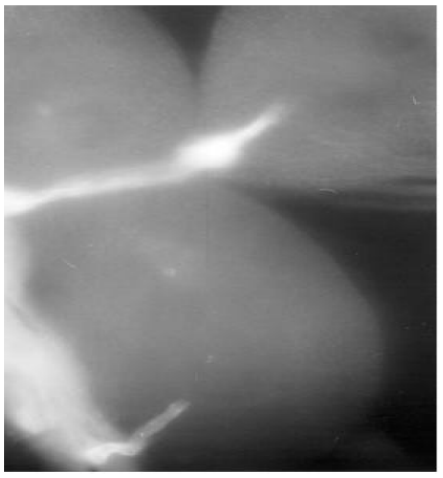

Fig. 1: Pollen tube entry into ovules of the cv. Invictus, 24 hours after pollination

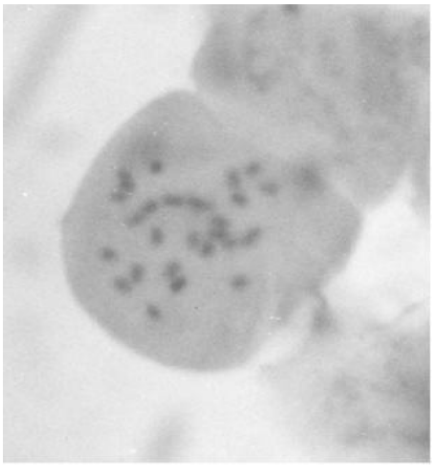

Fig. 2: A cell carrying 26 chromosomes in the plant number four

seedling development was rapid following germination and despite being similar to ordinary tomato seedling development, morphology of the developing seedlings differed from the characteristics of ordinary tomato seedling growth in that stem and cotyledon leaves were thicker and lighter green and true leaves had more lobes. Fruit set and ovule development varied for the cv. Invictus from 0, 17.9 to 32.4% for the three different pollination dates. This was similar to the results obtained from *Lycopersicon esculentum* x *Solanum lycopersicoides* crosses when the fruit set varied from the first year to the second. Flowers pollinated with *Solanum lycopersicoides* pollen set fruit 19.9 to 38 % for the first and second years, respectively. Similarly, in the first year, while 97 embryos from 38 fruits were obtained, 397 embryos were obtained from a total of 76 fruits in the second year (Chetelat *et al*., 1997). Regarding the number

Table 1: Fruit set and ovule culture from the cultivars pollinated with *Solanum sisymbriifolium* Lam. (Pollination date April 2001)

Parent	Pollination date (dd mm^{-1})*	Total no. of pollinations	Total fruit set (% fruit set)	No. of emptyfruits
Invictus	4/4	37	12 (32.4)	7
SC2121	6/4	26	-	-
Elif	6/4	23	-	-
Fantastik	7/4	34	-	-
Sagit 146	8/4	22	-	-
Gokce	8/4	29	-	-
Invictus	9/4	35	-	-
Y-18-90	10/4	31	-	-
TOTAL		237	12	7

*: day/month of the year 2001

of ovules and developing fruits, a similar instability was observed in *L. peruvianum* crosses (Doganlar *et al*., 1997; Egashira *et al*., 1999).

Although cross compatibility can be considered as the main factor for the instability, pollen viability may have also played an important role. The instability may have been the result of environmental factors or mechanical damages to the style during emasculation or pollination. A tomato flower's style is tender in texture and can easily be damaged or broken from its base at its attachment point to the ovary. During pollination, even a little extra pressure on the stigma may result in style breakage. Such styles do not permit pollen tube development into the ovary and soon wither, contributing to lowering the rates of fruit set.

In our study, the fact that pollen tubes developed down the style, entered the ovary and fruits developed in most parental combinations, ovules developed only in the cvs. Invictus and Sagit 146 which can be attributed either to the induction of parthenocarpy by *Solanum sisymbriifolium* Lam. pollination or embryo breakdown early in the development in the probable intergeneric background. Stevens and Rick (1994) observed parthenocarpic fruit set in crosses between *Lycopersicon esculentum* x *Lycopersicon pimpinellifolium*. Also, cv. Red Cherry x cv. Bonny Best cross combination resulted in parthenocarpic fruit development in higher temperatures, whereas developing fruits set seeds only in temperate growing conditions. In *Lycopersicon esculentum* x *Lycopersicon pimpinellifolium*, crosses, however, despite the abortion of the embryos within 2-4 weeks of pollination, fruits continued developing to the full size (Ho and Hewit, 1994). The above suggests that in *Lycopersicon esculentum* x *Solanum sisymbriifolium* Lam. crosses, in order to obtain a progeny, whether haploid, dihaploid or hybrid, early abortion of the embryos must be taken into account and an earlier embryo rescue schedule should be considered. In addition, availability of temperate growing temperatures before and after fruit set may be significant and high daytime temperatures may

Table 2: *In situ* pollen tube development and fruit set in cultivars pollinated with *Solanum sisymbriifolium* Lam. (Pollination date: May 2001)

Parent	Date of pollination	Total no. of pollination	Total fruit set (% fruit set)		In situ pollen tube development		No. of ovules cultured
					24 hours	48 hours	
Falcon	6/5	28	0	(0)	+	+	-[3]
Gokce	6/5	34	3	(8.8)	+	+	-
Elif	7/5	29	2	(6.8)	?[1]	+	-
Sagit 146	9/5	36	4	(11.1)	+	+	65[4]
Invictus	9/5	39	7	(17.9)	+	+	-
SC2121	10/5	23	2	(8.6)	+	+	-
Y-410-F1	10/5	21	0	(0)	-[2]	-[2]	-
Y-18-90	11/5	32	8	(25)	-[2]	-[2]	-
Total	242		24				

1: data not available
2: pollen tubes developed through to the middle of the style in both cases
3: ovules to be cultured were not available both for the cv. Falcon for which fruit development did not occur and for the other pollination combinations in which fruits did not contain ovules
4: All the cultured ovules were lost due to contamination following four days in culture.

have also been a factor in our experiments in the development of parthenocarpic fruits as the city of Adana, where the experiments were carried out, is located in a considerably warmer area.

In the chromosome counts of five plants a total of 12 cells with 24, 25 and 26 chromosomes were determined. The chromosome numbers remained stable for each root tip and the plant studied. The progeny produced by Chambonnet (1996) between *Lycoperscion esculentum* x *Solanum sisymbriifolium* were fertile and carried the same chromosome number as the parents, i.e. 24. The author also observed cells with 12 chromosomes which is unlike our study where not a single haploid chromosome cell was observed. The chromosome numbers determined were 24, 25 and 26 (Fig. 2). It may be that the progeny carrying the exact and complete number of chromosomes of tomato, i.e. 24, are doubled haploids. The chromosome number doubling may have been induced by the alien pollen, i.e. of *Solanum sisymbriifolium*, determination of which requires further work.

The progeny with the extra chromosomes, however, suggests that the plants may have been trisomic or tetrasomic. Although previous reports on *Solanum melongena* and *Solanum sisymbriifolium* and *Lycopersicon esculentum* and *Lycopersicon peruvianum* pollinations resulted in a progeny with 24 chromosomes (LanZhuang and Adachi, 1996; Bletsos *et al*., 1998), in a different *Lycopersicon esculentum* and *Lycopersicon peruvianum* hybridization experiment the progeny obtained resulted in chromosome numbers varying from diploid to triploid. It was thought that the extra chromosomes could be tolerated by the hybrid background of the progeny and tomato plants could tolerate up to three extra chromosomes and the number tolerated could rarely be four (Quiros, 1991).

Genotypic effect is significant in the haploid production via wide hybridization as in the cases of *Triticum aestivum* with *Zea mays* (Bitsch *et al*., 1998) and *Penisetum americanum* (Matzk and Mahn, 1994) and triticale with *Zea mays* (Wedzony *et al*., 1998). It is already known that pollinations onto *Lycopersicon* results in varying degrees of fruit set, ovule and embryo development depending on the parental combinations. The fact that only one *Solanum sisymbriifolium* genotype as the male parent in our study may provide limited information regarding the effects on the induction of *Lycopersicon* haploids. Therefore, the performance of other *Solanum sisymbriifolium* genotypes in combination with the varying genotypes of *Lycopersicon esculentum* remains to be tested.

Results, in general, indicate that *Lycopersicon esculentum* can be crossed with *Solanum sisymbriifolium* with a certain degree of success and further investigation is needed in order to determine full potentials of this approach in tomato breeding.

References

Anonymous, 2002. FAO Website http://apps.fao.org/cgi-bin/nph-db.pl?subset=agriculture.

Bitsch, C., S. Gröger and T. Leley, 1998. Effect of parental genotypes on haploid embryo and plantlet formation in wheat x maize crosses. Euphytica, 103: 319-323.

Bletsos, F.A., D.G. Roupakias, M.L. Tsaktsira, A.B. Scaltsoyjannes and C.C. Thanassoupoulos, 1998. Interspecific hybrids between three eggplant (*Solanum melongena* L.) cultivars and two wild species (*Solanum torvum* Sw. and *Solanum sisymbriifolium* Lam.). Plant Breeding, 117: 159-164.

Chambonnet, D., 1996. Essais d'haploidisation de la tomate. In: Rapport D'Activite 1995-1996 Station D'Amelioration Des Plantes Maraicheres D'Avignon-Montfavet, pp: 84-85.

Chetelat, R.T., P. Cisneros, L. Stamova and C.M. Rick, 1997. A male-fertile *Lycopersicon esculentum* x *Solanum lycopersicoides* hybrids enables direct backcrossing to tomato at the diploid level. Euphytica, 95: 99-108.

Chlyah, A., H. Taarji and H. Chlyah, 1990. Tomato (*Lycopersicon esculentum* L.) Anther culture and induction of androgenesis. In: Haploids in Crop Improvement I. Biotechnology in Agriculture and Forestry 12, Bajaj, Y.P.S. (Ed.), Springer-Verlag: Berlin, pp: 442-457.

Doganlar, S., A. Frary and S.D. Tanksley, 1997. Production of interspecific F1 hybrids, BC1, BC2 and BC3 populations between *Lycopersicon esculentum* and two accesions of *Lycopersicon peruvianum* carrying new root-knot nematode resistnce genes. Euphytica, 95: 203-207.

Egashira, H., R. Ogawa, H. Kano, T. Tanisaka and S. Imanishi, 1999. Pistillate-parental differences and the ability to produce interspecific hybrids between *Lycopersicon esculentum* and 'peruvianum-complex' species (*L. peruvianum* and *L. chilense*). Plant Breeding, 118: 253-258.

Foolad, M.R. and G.Y. Lin, 2001. Heritability of early blight resitance in a *Lycopersicon esculentum* x *Lycopersicon hirsutum* cross estimated by correlation between parent and progeny. Plant Breeding, 120: 173-177.

Gradziel, T.M. and R.W. Robinson, 1991. Overcoming unilateral breeding barriers between *Lycopersicon peruvianum* and cultivated tomato, *Lycopersicon esculentum*. Euphytica, 54: 1-9.

Ho, L.C. and J.D. Hewit, 1994. Fruit Development. In: The Tomato Crop, A Scientific Basis for Improvement, Atherton, J.G. and J. Rudich. (Eds.), pp: 201-239.

Hougas, R.W., S.J. Peloquin and A.C. Gabert, 1964. Effect of parent and polinator on the frequency of haploids in *Solanum tuberosum*. Crop Sci., 4: 593-595.

Kalloo, G., 1993. Tomato *Lycopersicon esculentum* Miller. In: Genetic Improvement of Vegetable Crops., Kalloo, G. and B.O Bergh. (Eds.), Pergamon Press: Oxford, pp: 645-666.

Kalloo, G. and M.K. Banerjee, 1990. Transfer of tomato leaf curl virus resistance from *Lycopersicon hirsutum* f. glabratum to *L. esculentum*. Plant Breeding, 105: 156-161.

Kasha, K.J. and K.N. Kao, 1970. High frequency haploid production in barley (*Hordeum vulgare* L). Nature, 225: 874-876.

LanZhuang, C. and T. Adachi, 1996. Efficient hybridization between *Lycopersicon esculentum* and *L. peruvianum* via 'embryo rescue' and *in vitro* propagation. Plant Breeding, 115: 251-256.

Matzk, F. and A. Mahn, 1994. Improved techniques for haploid production in wheat using chromosome elimination. Plant Breeding, 113: 125-129.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum, 15: 473-497.

Quiros, C.F., 1991. *Lycopersicon* Cytogenetics. In: Chromosome Engineering in Plants: Genetics, Breeding, Evolution, Part B, Development in Plant Genetics and Breeding 2B, Tsuchiya, T. and P.K. Gupta, (Eds.), Elsevier: Amsterdam, pp: 119-138.

Rick, C.M., J.W. DeVerna, R.T. Chetelat and M.A. Stevens, 1987. Potential contributions of wide crosses to improvement of processing tomatoes. Acta Hort. (ISHS), 200: 45-56.

Stevens, M.A. and C.M. Rick, 1994. Genetics and Breeding. In: The Tomato Crop, A Scientific Basis for Improvement. J.G. Atherton, J. Rudich (Eds.), Chapman and Hall, London, pp: 35-109.

Vidavski, F., S. Leviatov, J. Milo, H.D. Rabinowitch, N. Kedar and H. Czosnek, 1998. Response of tolerant breeding lines of tomato, *Lycopersicon esculentum*, originating from three different sources (*L. peruvianum*, *L. pinpinellifolium* and *L. chilense*) to early controlled inoculation by tomato yellow leaf curl virus (TYLCV). Plant Breeding, 117: 165-169.

Wedzony, M., I. Marcinska, A. Ponitka, A. Slusarkiewicz-Jarzina and J. Wozna, 1998. Production of doubled haploids in triticale (x *Triticosecale* Wittm.) by means of crosses with maize (*Zea mays* L.) using picloram and dicamba. Plant Breeding, 117: 211-215.