Iran Agricultural Research 14:139-156 (1995)

A COMPARISON BETWEEN *IN VITRO* CULTURE OF TWO TOMATO PARENTAL LINES AND THEIR RECIPROCAL HYBRIDS

M. ETEHADNIA AND M. KHOSH-KHUI[1]

Department of Horticulture, College of Agriculture, Shiraz University,

Shiraz, Iran.

(Received: February 22, 1995)

ABSTRACT

Two tomato parental lines, Red Cloud (RC) and a local cultivar (L), were reciprocally crossed. The regenerating abilities of the parental lines and the hybrids were compared through shoot-tip culture. Prior to *in vitro* culture, some morphological characteristics of the two tomato reciprocal hybrids (RC×L) and (L×RC) and their parents were evaluated. In *in vitro* culture, the effects of five concentrations of benzyl adenine (BA) and kinetin (K) and four cultures (one original and three subcultures) were examined on the tomato parental lines and the hybrids. The effects of indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) concentrations on root initiation of two parental lines and the hybrids were also evaluated. Heterosis was observed in the hybrids compared to the parents for vigor, yield and some qualitative traits. BA affected callus and shoot formation more than K. Local×RC had the highest callus fresh weight on media containing BA and K whereas RC×L hybrids produced more shoots compared with L×RC and the parents.

1. Former Graduate Student and Professor, respectively.

Both hybrids needed lower levels of BA than did the parents and required lower levels of K compared with RC parent. Among various lines, L×RC hybrids heeded the lowest levels of BA and K. Number of shoots in RC was decreased by subcultures. However, shoot numbers in local parents and two hybrids increased in subsequent subcultures. Generally, NAA produced more fresh and dry root weight in two parental lines and their hybrids. The growth regulators IBA and NAA induced long, thin, white and short, thick, brown roots, respectively. All four lines produced suitable roots on media containing IBA, and were transferred to soil successfully.

تحقیقات کشاورزی ایران

۱۴: ۱۳۹-۱۵۶ (۱۳۷۴)

مقایسه ای بین ریزافزایشی دو لینه والد گوجه فرنگی و دورگه های آنها

معصومه اتحادنیا و مرتضی خوشخوی

به ترتیب دانشجوی سابق کارشناسی ارشد و استاد بخش باغبانی دانشکده کشاورزی دانشگاه شیراز ، شیرا ز ، ایران.

چکیده

دورگه گیری بین دو لینه والد گوجه فرنگی ردکلود (RC) ومحلی (L) بصورت دو طرفه انجام شد. شاخه زایی دو والد و دورگه های آنها به روش کشت نوک شاخساره مقایسه شد. تعدادی ا ز صفات ظاهری والدین و دورگـه هـای حـاصل RC× L و L× RC پیـش ا ز کشـت درون شیشـه ای مورد مقایسه قرارگرفتند . در کشت درون شیشه ای، اثرات پنج غلظت بنزیل آدنین (BA) و کینتین (K) و چهار کشت (یک کشت اصلی وسه زیـر کشـت) روی والدیـن و دورگـه هـای آنهـا مـورد

بررسی قرار گرفت . همچنین اثرات اسید ایندول بوتیریک (IBA) و اسید نفتالن استیک (NAA) روی ریشه زایی والدها و دورگه های آنها ارزیابی شدند. دورگه های به وجود آمده ، از نظر قدرت رشد میزان محصول وصفات کیفی نسبت به والدین برتربودند. بطور کلی ، بنزیل آدنین درمقایسه با کینتین تاثیر بیشتری بر تولید پینه و شاخه زایی داشت . دورگه L×RC ، بیشترین وزن پینه تازه را در محیط کشت های دارای K و BA داشت. دورگه RC×L نسبت به L×RC و همچنین والدین تعداد شاخساره بیشتری تولید کرد. درمجموع ،دورگه هاکمتر از والدین به BA نیاز داشتند . همچنین دورگه ها ،K کمتری از والد RC لازم داشتند. از بین لینه ها دورگه L×RC نیاز به حد اقل میزان K و BA داشت . مقایسه زیرکشت ها نشان داد که تعداد شاخسارهای دورگه و والد محلی درکشت های بعدی افزایش می یابد ولی رقم RC این ویژگی رانشان نداد. NAA باعث تولید وزن تر و خشک بیشتری در والدها و دورگه های آنها گردید. اکسین IBA ریشه های بلند و نازک وسفید و NAA ریشه های کوتاه ضخیم و قهوه ای تولید کرد. والدها و دو رگه های آنها در محیط کشت IBA ریشه زایی مناسبی داشتند و با موفقیت به خاک انتقال یافتند.

INTRODUCTION

Using heterosis is a basic and highly-effective breeding method for developing early, high-yielding, uniform tomato cultivars. Additionally, the method combines a number of other valuable economic characters (4, 8). Due to segregation in seed multiplication of F_2 generation, breeders always try to propagate F_1 tomato plants vegetatively. Tissue culture is a method of

vegetative propagation for rapid multiplication of plants and shoot-tip culture is widely used for conservation of F_1 tomato plants.

Hussey (10) examined the behavior of tomato shoot apexes excised from the parent plant with varying amount of sub-apical tissues cultured under different cultural conditions. Kartha *et al.* (13) regenerated plants in high frequency from shoot-tip cultures for elimination of systemic viral infection. Novak and Maskova (16) studied the effect of phytohormones on growth and development of apical shoot-tip of tomato and determined the possibilities of clonal plant propagation for genetic and selection purposes. Deng *et al.* (6) obtained a large number of plantlets from shoot-tips of tomatoes cultivated on Murashige and Skoog (MS) medium with various growth regulators. Plants were hardened and transplanted into the soil. Compared with the donor cultivars, regenerated plants had 100% increase in yield.

This investigation was undertaken to study a means for conserving heterosis in tomato F_1 plants through shoot-tip culture by comparing shoot proliferation in two tomato parental lines and the reciprocal hybrids.

MATERIALS AND METHODS

Hybridization

Seeds of parental cultivars were planted in a soil consisting of 1:1:1(v/v) field soil, sand and peat moss in a greenhouse at 20±4 °C temperature and 60 to 80% RH. Before reciprocal crossing, all opened flowers of an appropriate female parent were picked off to avoid self-fertilization. The floral buds selected for crossing were emasculated. All non-emasculated flowers were removed from the female plants. The emasculated flowers were tagged and covered with a paper bag untill pollination which was performed two days later .

To perform artificial cross-pollination, anthers of opened flowers were picked off from the male parent, placed on the style of emasculated flowers and covered with a paper bag. The bags were removed four days later. Fruits ripened eight and twelve weeks after hand pollination for RC×L an L×RC, respectively. Fruits were crashed and kept for four days at 25° C for fermentation. Mature seeds were extracted, washed and dried. Seeds were stored at 25° C.

After planting in the greenhouse and prior to *in vitro* comparison of F_1 progenies with their parental lines, the following characteristics were recorded: leaf length, time of flowering, number of flowers per plant, time of maturity, yield per plant, ascorbic acid content, titratable acidity, total soluble solids and pH.

Explant Preparation and Culture

Eight weeks after planting in the greenhouse, 1-cm long shoot-tips were picked for *in vitro* culture. To reduce surface contaminants, shoots were first washed in tap water and then were surface sterilized in 10% Chlorox (5.25% sodium hypochlorite) solution for ten minutes and rinsed three times with sterilized water before placing in culture. Shoot-tips about 0.5 cm long were removed and asceptically transferred to the culture media.

The medium used for callus production and shoot proliferation consisted of Murashige and Skoog (MS) salts (15) supplemented with various concentrations of benzyl adenin (BA) and kinetin (K). On the basis of preliminary experiments, 1.00, 1.25, 1.50, 1.75, and 2.00 mg l^{-1} of BA and 2.00, 2.50, 3.00, 3.50, and 4.00 mg l^{-1} of K were selected for shoot proliferatioh.

The rooting medium consisted of MS medium supplemented with IBA or NAA at various concentrations. On the basis of the preliminary experiments 0.25, 0.50, 1.00, and 2.00 mg l^{-1} of either IBA or NAA were selected

Cultures were kept at 26± 2 °C under a 14-hr photoperiod of 1800 lux light intensity emitted by two cool-white fluorescent lamps.

Subculturing

The callus produced after four weeks from a shoot-tip was divided and cultured on the same medium used for shoot production. New shoots were subcultured for more shoot multiplication.

Rooting of Shoots and Transfer to Soil

After three subcultures, propagules were transferred to the rooting medium. Rooted plantlets were transferred to pasteurized soil after four weeks . Soil pasteurization was performed by autoclaving at 121 °C and 1.5 kg cm^{-2} steam pressure for 1 hr. Soil was a mixture of 1:1:1 (v/v) field soil, sand, peat moss and vermiculite The plants were first irrigated with sterilized water and the pots were placed under laboratory conditions in a container having an adjustable air opening. The plants were gradually adapted to normal conditions and after two weeks were transferred to the greenhouse.

All experiments were carried out in completely randomized design with at least 10 replications and the means were compared using Duncan's new multiple range test.

RESULTS AND DISCUSSION

The Evaluation of Parental Lines and the Reciprocal Hybrids

Phenotypic differences were observed between the parental lines and their reciprocally crossed F_1 progenies for vigor, time of flowering and maturity, number of flowers, yield and qualittaive traits. Hybrids had longer leaves

than their parents. This could be attributed to the heterosis. Similar findings were reported by Sankina and Tropina (21) for plant weight.

The intermediate flowering time observed in hybrids of this study is in accordance with the results obtained by Gibrel *et al.* (8). They reported that when early and late cultivars were crossed, most hybrids flowered at dates intermediate to the parents.

Obtaining more flowers in RC×L hybrids is consistent with the results of several investigators (e.g. 2). Local×RC had the same number of flowers as the higher flower producing parent. Bearing more flowers in RC×L than L ×RC might be due to the cytoplasmic inheritance. In this study, when RC was the female parent, F_1 plants produced more flowers.

The fruit earliness of F_1 hybrids, compared to late ripening parent observed in this investigation, has also been reported previously. For example, Gibrel *et al.* (8) crossed early and late ripening cultivars and observed that the maturity of F_1 hybrids was usually intermediate between the two parents.

Hybrids, paricularly RC×L , had higher yields compared to the parents. Similar results have been reported by others (e.g. 20).

The highest ascorbic scid content, titratable acidity and soluble solid content was obtained in RC×L hybrids. Similar findings have been obtained in a previous investigation (20).

The lowest level of fruit juice pH was obained in RC×L hybrids. It has been previously shown that fruit juice from hybrids usually have significantly lower pH as compared with the parental lines (4).

Shoot-tip Culture

Callus initiation. After 2 to 3 weeks of *in vitro* culture, green compact callus was produced at the base of explants of both parents and their hybrids. However, callus color changed to brown if it was not subcultured for a given

period of time. Kartha *et al.* (13) reported that tomato shoot apical meristems produced callus after 7 days, and Novak and Maskova. (16) observed compact callus from shoot-tips of tomato after 10 days of culture. These differences in time, might be due to cultivar genotypes, age of explants and or the composition of culture media.

The variations observed in callus growth of parents and their hybrids were dependent on the type and concentration of growth regulators. Frankenberger *et al.* (7) evaluated 21 tomato genotypes and observed variations in callus production.

The effects of BA and K on callus fresh weight of two parental lines and their hybrids are shown in Table 1. All lines produced more callus

Table 1. Mean callus fresh weight (g) of parental lines and their hybrids on most suitable concentrations of BA and K (the concentrations used are shown in parentheses in mg l^{-1}).

Growth regulators	RC	L	RC × L	L × RC
BA	2.08 aB† (2.00)	1.24 aC (1.75)	2.33 aB (1.50)	2.78 aA (1.00)
K	1.71 bB (4.00)	0.87 bC (3.00)	1.96 bB (2.50)	2.40 bA (2.00)

† Means followed by the same letters (capital letters for rows and small letters for columns) are not significantly different at 1% level of probability using DNMRT.

fresh weight on the medium supplemented with BA than K. Novak and Maskiva (16). reported that BA had an inhibitory effect on root initiation. Such an effect was not observed in this study. Comparisons between callus fresh weight of two parents indicated that RC had greater callus formation ability than L. Other L×RC hybrids had significantly higher callus regenerating ability than their parents and the L×RC hybrid. The effect of

genotype on callus fresh and dry weight and diameter has been reparded in a previous investigation (3).

Both hybrids needed lower hevels of BA than their parents and lower levels of K compared to RC parent. Lower phytohormone requirements of hybrids than their parents could be attributed to the effects of heterozygosity.

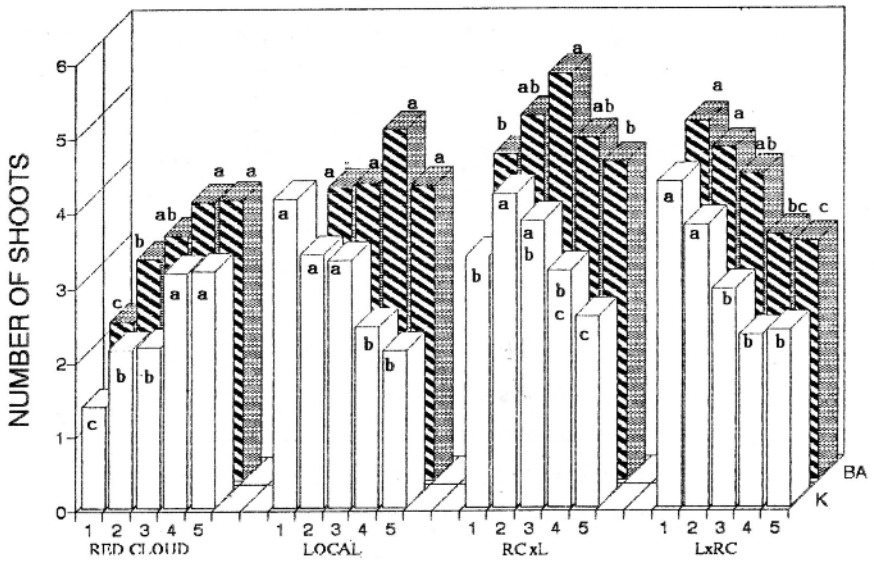
Shoot proliferation. Shoot formation in two parental lines and their hybrids was different at various concentrations of growth regulators. Observing the leaf sections of 12 tomato cultivars showed that morphogenetic responses were cultivar dependent and exhibited differences over a range of growth regulators (14).

The effects of different concentrations of BA and K on the two parental lines and their hybrids are shown in Fig. 1. Generally, both parents and hybrid explants produced higher numbers of shoots on the media containing BA than K. Izadpanah and Khosh-Khui (11) working on RC, Cal-j and Petomech, reported that BA induced more shoot proliferation than K in all cultivars.

The differences between mean shoot number of parents and their hybrids on media containing BA and K were significant. Responses of different genotypes to growth regulators were different as evaluated by the number of shoots. Shoot proliferation rate of the L parent was higher than RC parent. In a similar experiment (17) , shoot forming capacity of hypocotyl explants of two parents and their hybrids, was compared and it was concluded that one of the parents always had a higher shoot forming capacity than the other.

In this study, RC which produced more callus weight than the L parent, was not superior in shoot forming capacity. Such differences were also noticed by Padmanabhan *et al.* (18).

Red Cloud×L hybrids formed more shoots than L×RC or the parents on both BA and K media. Shoot production of RC×L hybrids is shown in Fig. 2.



	BA(mg l^{-1})	K(mg l^{-1})
1:	1.00	2.00
2:	1.25	2,50
3:	1.50	3.00
4:	1.75	3.50
5:	2.00	4.00

Fig. 1. Comparison between number of shoots produced by two parental lines and their hybrids on different concentrations of BA and K. Means followed by the same letters are not significantly different at 1% level of probability using DNMRT.

Fig. 2. Shoots obtained after four weeks for a RCxL shoot-tip cultured on a MS medium supplemented with 1.5 mg l^{-1} BA.

In spite of this, shoot forming ability of L×RC hybrids was more than the two parents in media containing BA. Heterosis in shoot forming capacity observed in two hybrids of the present study was also described by Ohki *et*

al. (17) for other cultivars. They carried out a screening test for two reciprocal hybrids and their parents and indicated that the hybrids showed heterosis with regard to shoot forming capacity.

Subcultures. Parents and their hybrids produced significantly different number of shoots when subcultured on the appropriate fresh media (Table 2). Number of shoots in RC was reduced in subcultures, but the L parent and the two hybrids showed successively increased number of shoots. This shoot reduction in RC may be due to low shoot forming capacity of this cultivar in comparison with the other parent and hybrids. Pamela *et al* .(19) studied effects of cytokinins on organogenesis of cultured cotyledons of several cultivars and subcultured them several times. They found that organogenesis decreased with subculturing but continued to occur with same treatments, particularly with IPA [N6-(2-isopentyl adenine)].

Rooting. Rooting was obtained by transferring the developed shoots onto MS medium supplemented with IBA or NAA after 2 weeks of cultures. Explants also produced root in shoot forming media in a longer time. Neccessity of using auxin for root formation in shoot-tip culture of tomato has been emphasized (11, 13, 14). Kurz *et al.* (14) reported that leaf explants of all cultivars rooted on a medium supplemented only with 0.20-2.00 mg l^{-1} IAA. Gunay and Rao (9) worked on propagation of hybrid tomato plants by using hypocotyl and cotyledon explants. They showed that when regenerated shoots failed to develop roots, rooting took place by transplanting the shoots to MS medium containing 0.5 mg l^{-1} IAA. Presence of high auxin level has been reported in tomato stem (5, 12). This might account for the formation of roots in tomato forming media. Kartha *et al.* (13) also reported that shoots diffe rentiated at all concentrations of cytokinins and produced a well-developed root system after 60 days.

Generallv, IBA induced long, thin and white roots and NAA short, thick and brown roots. High concentrations of IBA (more than 2.0 mg l^{-1})

Table 2. Mean shoot number of parental lines and their hybrids in original culture and subcultures (data are averaged over different concentrations of each growth regulators).

Growth regulators	Cultures	R C	L	RC × L	L × RC
BA	Original	4.2 a†	3.2 b	3.7c	2.3c
	Subculture 1	3.2 b	3.8 ab	4.1 bc	3.7 b
	Subculture 2	2.7 c	4.2 a	4.8 b	4.3 b
	Subculture 3	2.6 c	4.3 a	6.5 a	5.6 a
K	Original	2.9 a	2.8 a	2.4 c	2.0 d
	Subculture 1	2.6 a	3.2 a	3.0 b	2.9 c
	Subculture 2	1.9 b	3.0 a	3.5 b	3.5 b
	Subculture 3	2.1 b	3.3 a	4.9 a	4.2 a

† In each column, means followed by the same letters are not significantly different at 1% level of probability using DNMRT.

produced thick, short and brown roots. The effects of IAA and IAA conjugates on tomato leaf disc morphogenesis were studied by Valeric *et al.* (22). who showed that the roots were shorter at higher auxin levels.

Mean root fresh and dry weights of parents and their hybrids are presented in Table 3. IBA had no effect on root fresh weight of the two parental lines and the hybrids, but showed significant effects on dry weight of the lines under the study. Both hybrids had the higher dry weight on IBA than parents.

Both fresh and dry weight means of parental lines and the hybrids were significantly affected by NAA. Root forming ability of the two parental lines and their hybrids are shown in Fig. 3.

Table 3. Mean fresh and dry weight of roots of parental lines and their hybrids on media containing IBA and NAA (data are averaged over differernt concentrations of each growth regulator).

Growth regulator	R C	L	RC × L	L × RC
		Fresh weight (g)		
IBA	0.32 aA†	0.32 aA	0.33 aA	0.36 aA
NAA	0.56 bB	0.56 bB	0.72 bA	0.77 bA
		Dry weight (g)		
IBA	0.05 aB	0.05 aB	0.06 aA	0.06 aA
NAA	0.06 bC	0.06 bC	0.07 bB	0.08 bA

† Means followed by the same letters (capital letters for rows and small letters for columns) are not significantly different at 1% level of probability using DNMRT.

ACKNOWLEDGEMENT

This research was supported in part by a grant from Shiraz University Research Council.

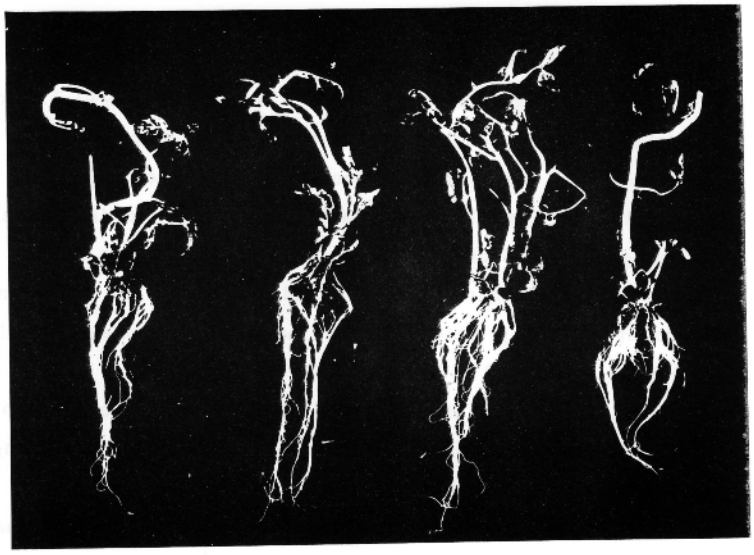


Fig.3. Root formation ability of two parental lines (A: Local, B: Red Cloud) and their hybrids (C: L×RC, D: RC×L) on MS + 0.5 mg l^{-1} IBA.

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