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Full Length Research Paper

The role of growth regulators, embryo age and genotypes on immature embryo germination and rapid generation advancement in tomato (*Lycopersicon esculentum* Mill.)

Naif Geboloğlu*, Süleyman Bozmaz, Mine Aydin and Perihan Çakmak

Department of Horticulture, Agricultural Faculty, Gaziosmanpaşa University, Tokat, Turkey.

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One of the most important problem of tomato breeders is lengthy seed to seed cycle in a breeding program. *In vitro* techiques provide a lot of advantages for breeders. The objective of this work was to determine the effect of growth regulators and immature embryo age on embryo germination and rapid generation advancement in different tomato genotypes. For this purpose, four different tomato genotypes were used. Immature embryos were derived from fruits 20, 24, 28, 32 and 36 days after pollination. Fruits were left on the plant to mature as control (conventional breeding cycle). MS, MS + 0.1 mg/l kinetin, MS + 0.1 mg/l IAA and MS + 0.1 mg/l kinetin + 0.1 mg/l IAA were used as growth regulators. Growth regulators and genotypes resulted in nonsignificant effect on germination rate and rapid generation advancement, but embryo age resulted in significant effect. Germination rate from immature embryos ranged between 55.22 and 100%. Twenty eight (28) and thirty two (32) days old embryos gave the best germination rate. Days from pollination to flowering, shortened between 53,36 and 63,96 days in comparison with the conventional breeding cycle. This study showed that using immature embryo technique in tomato breeding offers more generation per year when compared to conventional breeding practice.

Key words: Growth regulators, immature embryo culture, embryo age, tomato, kinetin, IAA, shortened breeding cycle.

INTRODUCTION

Tomato is one of the most important vegetable crops with worldwide production of 141,40 million tons from 4,98 million ha in 2009 (Anonymous, 2009). Cultivated tomatoes suffer from many biotic and abiotic stress factors which also limit tomato production. Resistance to these stress factors is the primary objective of many tomato breeding programs (Chen and Adachi, 1992). Cultivar development using conventional methods takes long time, assuming one generation annually. To increase breeding efficiency and shorten the time, advanced methods and techniques are regularly incorporated in tomato breeding programs. *In vitro* techniques are important tools for modern cultivar improvement programs (Barbano and Topolesky, 1984; Moghaieb et al., 1999; Taji et al., 2002; Bhatia et al., 2004). In conventional tomato breeding, a field tomato crop requires at least 3 to 4 months from sowing to mature seeds extraction before these can be reseeded for the next generation. Of this time, seed development from pollination to maturity takes at least 2 months. This period could be reduced by techniques of *in vitro* immature embryo culture. Immature embryo culture is the cultivation of zygotic embryos excised from ovules and seeds under aseptic conditions in growth regulators. It represents an important milestone with efforts to identify the requirements essential for continued growth, differentiation and morphogenesis of embryos (Raghavan, 2003). *In vitro* zygotic embryo culture depends on many factors, most important of

*Corresponding author. E-mail: naif@gop.edu.tr or naifgebologlu@hotmail.com. Tel: +903562521616/2112. Fax: +903562521488.

which are: genotype, explant, embryo age, composition of basic growth media, growth regulators, light intensity and quality, photoperiod, temperature and endogenous factors (Rinaldi, 1999; Umehara et al., 2007; Polanco and Ruiz, 2001). One of the important factors for a high success in zygotic embryo culture is the age of immature embryos. Demirel and Seniz (1997) suggested that 25 days after pollination (DAP) was the minimum period for equilibrium between the number of recovered embryos and their developmental stage for securing functional plant from immature tomato seeds. The other important factor for a high success in zygotic embryo culture is the composition of culture growth regulators. Growth regulators components have proved to influence the efficiency of embryo rescue techniques in many crops (Polanco and Ruiz, 2001). Bhattarai et al. (2009) indicated that the best culture growth regulators were modified full strength Murashige Skoog (MS) salts supplemented with 0.1 mg/l IAA, 0.5 mg/l IBA, 0.5 mg/l GA_3 and 2% sucrose for tomato embryo culture.

The aim of this study was to evaluate the influence of different growth regulators, embryo age and genotypes on zygotic embryogenesis and determine the effect on shortening breeding time in tomato.

MATERIALS AND METHODS

F_2 generation of Alida F1, Newton F1 and Astona F1 (indeterminate tomato cultivars) and T-01 (local determinate tomato genotype) were used. Four to five leafy seedlings were planted in unheated greenhouse with 25 to 32°C daytime temperature and 75 to 80% relative humidity in Tokat/Turkey. Plants were grown with 100 cm row spacing and 40 cm plant spacing. Cultural practices such as fertilization, irrigation, pruning, training, disease and pest control, etc, were applied according to Hickman (1998). Fruits were harvested 20, 24, 28, 32 and 36 days after self pollination and stored in a dark room at 24 ± 2°C for 24 h. Stored fruits were sterilized with 70% ethanol for 3 to 4 s and in 5% sodium hypocloride for 20 min and washed twice with sterile distilled water. Sterilized fruits were transferred into a sterile flow cabin for embryo derivation. Some fruits were left on the plants to quantify the generation time with normal ripening and harvested when they reached physiological maturity. Embryos at different developmental stages were excised from sterile fruits and transferred to the following basic culture growth regulators. MS growth regulators were supplemented with 30 gl^{-1} sucrose and 8 gl^{-1} agar (Murashige and Skoog, 1962). The pH was adjusted to 5.7 before autoclaving. Three plant growth regulators, 0.1 mg/l kinetin (Kin), 0.1 mg/l IAA and 0.1 mg/l kinetin + 0.1 mg/l IAA (Kin + IAA) were used. MS growth regulators supplemented with 30 gl^{-1} sucrose and 8 gl^{-1} agar were used as control media. Growth regulators were filtered and sterilized, mixed with the autoclaved media, and dispensed at 20 ml per 60 ml Petri dish. For every maturity-class, 40 embryos were transferred into Petri dishes. All cultures were incubated in the dark for 36 h and main-tained at 25 ± 1°C, 65 ± 2% relative humidity, under the 15 000 lux light intensity and a 16-h photoperiod in a controlled growth room. Rooted and germinated plantlets (~15 days after they were transferred into Petri dishes) were transferred into sterile glass tubes including MS + 30 gl^{-1} sucrose + 8 gl^{-1} agar. Plantlets (seedlings) reached 4 to 5 true leafy stage ~7 to 10 days after they were transferred into tubes. Seedlings were transferred into 8 L pots containing peat. They were grown to flowering. Days from inbreeding to flowering were recorded. In the control plants, seeds were extracted from normal vine ripened fruits and they were sown into pots.

Statistical analysis

The effects of growth regulators, embryo age and genotypes were analysed using ANOVA, with means seperated by the Duncan test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Germination rate

Germination from immature embryos ranged between 55.22 (20 days old embryos of T-01 genotype on MS + Kin) and 100% (in nine applications) in this study (Table 1). Germination rate ranged between 84.92 and 87.56% in genotypes, and 83.19 and 87.92% in growth regulators (Figure 1). The lowest germination rate was obtained from 20 day-old embryos (Figure 2). These embryos were derived from fruits under microscope because of their small size. Some of these embryos were injured due to their small size. Germination was not obtained from injured embryos. Consequently, success rate was low in 20 days-old embryos. Generally, germination rate increased steadily from 20 day-old embryos up to 32 days-old embryos. The highest germination was obtained from 28 and 32 day-old embryos (Figure 2). Embryo age has been the most effective factor in germination rate. Effect of genotypes and nutrient growth regulators were found to be statistically insignificant on germination rate, while embryo age was significant ($P \leq 0.01$) (Table 1). In our study, the lowest germination rate was obtained from MS + Kin and the highest from MS.

A number of studies have shown similar results with our study. Aragao et al. (2002) indicated that the best results were obtained from 30 days-old embryos, Demirel and Seniz (1997) reported that 25 days after pollination was the minimum period for germination. In our study, minimum germination was obtained from 20 days-old embryos and the best results were obtained from 28 and 32 days-old embryos. Generally, when embryo age increased, germination rate increased, too. Germination was realized to be 100% in 9 different combinations. Bhattarai et al., (2009), stated that the minimum embryo age was 10 DAP, and germination rate was 61% for 10 DAP and 90% for mature seeds. According to Gubis et al. (2003), germination rate ranged between 86 and 100%, and genotype had no effect on germination. Plana et al. (2006) indicated that shoot regeneration rate was 100% in immature embryo culture.

Rapid generation advancement

The time from pollination to flowering ranged between

Table 1. Germination rate for genotype, embryo age and growth regulators (%).

Genotype	Embryo age	Growth regulator				Mean	
		MS	MS + Kin	MS + IAA	MS + Kin + IAA		
Alida	20	65.26	60.24	62.75	65.26	63.38	87.02
	24	82.83	77.81	85.34	82.83	82.20	
	28	97.89	92.87	100.00	97.89	97.16	
	32	97.89	87.85	100.00	100.00	96.44	
	36	100.00	92.87	95.38	95.38	95.91	
Newton	20	62.75	57.73	60.24	60.24	60.24	85.19
	24	85.34	77.81	85.34	82.83	82.83	
	28	95.38	87.85	92.87	92.87	92.24	
	32	100.00	95.38	97.89	95.38	97.16	
	36	97.89	90.36	92.87	92.87	93.50	
Astona	20	70.28	60.24	62.75	65.26	64.63	87.56
	24	82.83	85.34	82.83	87.85	84.71	
	28	95.38	92.87	97.89	95.38	95.38	
	32	97.89	100.00	97.89	100.00	98.95	
	36	95.38	92.87	95.38	92.87	94.13	
T-01	20	57.73	55.22	60.24	60.24	58.36	84.92
	24	82.83	77.81	80.32	82.83	80.95	
	28	95.38	87.85	90.36	92.87	91.62	
	32	100.00	97.89	100.00	95.38	98.32	
	36	95.38	92.87	95.38	97.89	95.38	
Means		87.92	83.19	86.81	86.17		

Statistical differences

Genotype	ns	Genotype x media (Figure 1)	ns
Media	ns	Media x embryo age (Figure 2)	**
Embryo age	**	Genotype x embryo age	*
Genotype x media x embryo age:	**		

*, Significant differences at level P ≤ 0.05; **, significant at level P ≤ 0.01; ns: not significant.

69.3 (20 days-old embryos of Alida on MS) and 128.6 days (32 days-old embryos of the T-01 on MS+IAA) (Table 2). It ranged between 127.6 (T-01 genotype) and 139.2 days (Newton genotype) in the control plants where the fruit was left on the plant for maturing (Figure 3). The shortest time was obtained from Alida genotype as 86.2 days; and the longest was from Newton as 98.2 days. It ranged from 90.4 days in MS and 93.2 days in MS + Kin + IAA. DAP to flowering was not significantly affected by the genotypes and growth regulators. The time from pollination to flowering, depending on the age of the embryos showed statistical significant differences (P ≤ 0.001) (Table 2). This time in 20, 24 and 28 days-old immature embryos was shorter than that of 32 and 36 days-old immature embryos (Figure 4). According to the findings in control plants, the time from pollination to flowering of immature embryos shortened as 63.63, 63.96, 54.47 and 53.36 days in Alida, Newton, Astona and T-01 genotypes, respectively (Figure 3).

Previous studies showed that embryo culture shortened the breeding cycle in many species (Pierik, 1997; Acebedo et al., 1997; Cravero and Cointry, 2007; Ochatt et al.,

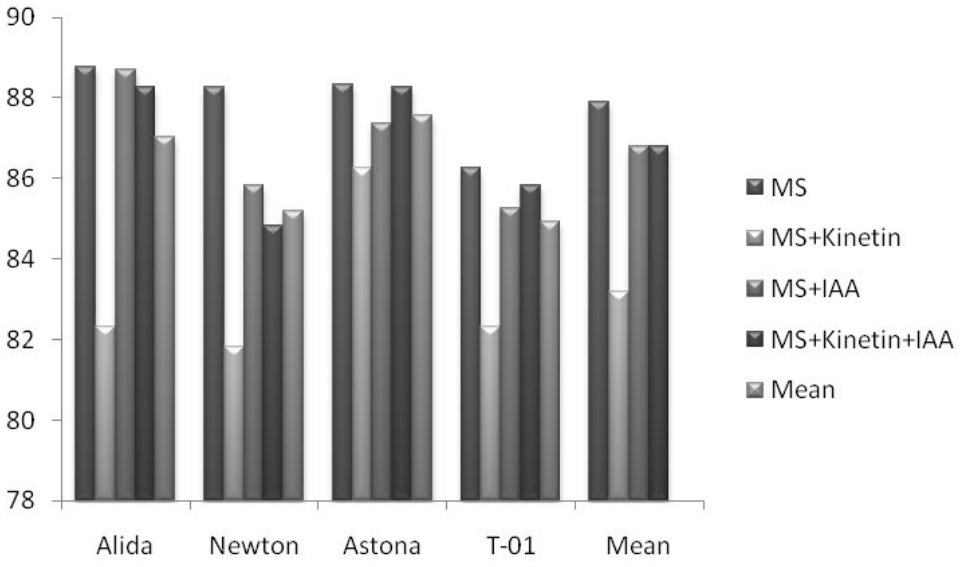

Figure 1. Germination rate for genotypes.

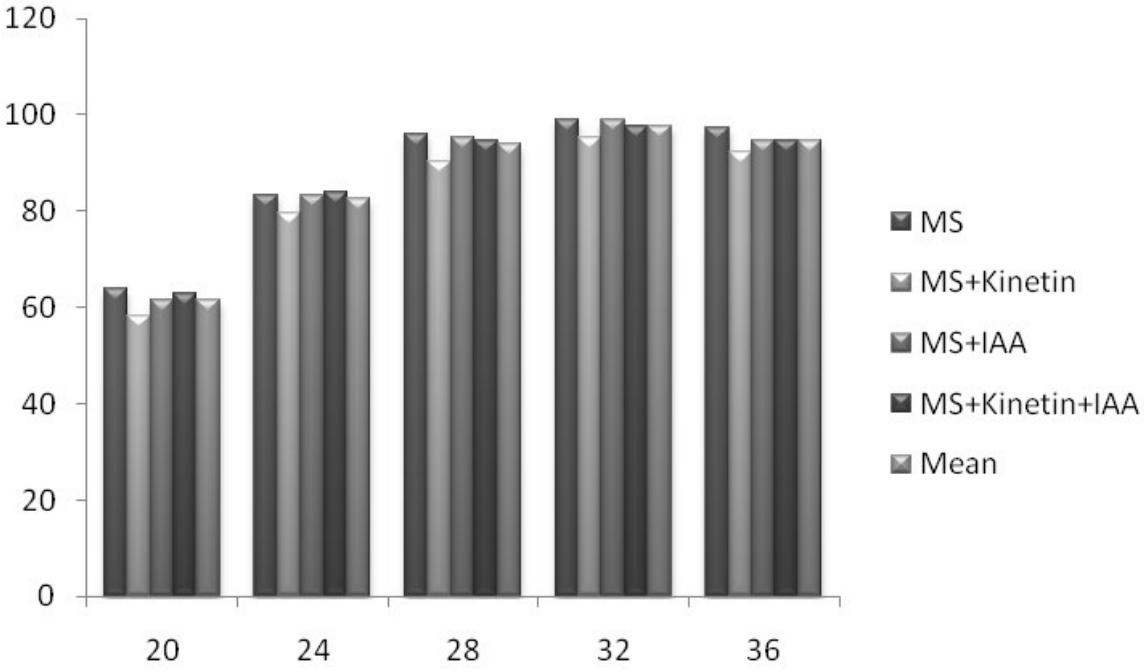

Figure 2. Germination rate for embryo age.

2002; Yuan et al., 2003). In our study, breeding cycle was shortened between 53.36 and 63.96 days in comparison with the controls.

Conclusion

The immature embryo culture technique germinated into new tomato plants from 20 days old embryos with a low success rate. The germination percentage reached 100% when the embryo age reached 28 to 32 days. Shoot germination rate was not effected by growth regulators or genotypes. Using the immature embryo culture provided an ad-vantage in rapid generation advancement in comparison with the conventional breeding practice. Immature embryo culture technique offered up to 3 generations, in contrast to conventional breeding systems which has maximum of 1 to 2 generations per year.

Table 2. The time from pollination to flowering for genotype, embryo age and growth regulators (days).

Genotype	Embryo age	*In vitro* media				Mean	
		MS	MS + Kin	MS + IAA	MS + Kin + IAA		
Alida	20	69.3	83.4	81.4	76.3	77.6	86.2
	24	74.3	74.3	74.3	75.3	74.6	
	28	81.4	81.4	83.4	83.4	82.4	
	32	96.4	96.4	96.4	96.4	96.4	
	36	100.5	98.4	99.4	101.5	99.9	
Newton	20	75.3	77.3	78.3	99.4	82.6	98.2
	24	81.4	79.3	79.3	83.4	80.9	
	28	97.4	98.4	98.4	97.4	97.9	
	32	116.5	116.5	115.5	117.5	116.5	
	36	104.5	119.5	104.5	123.6	113.0	
Astona	20	75.3	75.3	76.3	78.3	76.3	92.9
	24	81.4	81.4	81.4	81.4	81.4	
	28	99.4	100.5	100.5	102.5	100.7	
	32	95.4	106.5	105.5	107.5	103.7	
	36	101.5	99.4	100.5	108.5	102.5	
T-01	20	77.3	79.3	79.3	79.3	78.8	92.5
	24	74.3	74.3	74.3	74.3	74.3	
	28	71.3	71.3	91.4	91.4	81.4	
	32	127.6	126.6	128.6	120.6	125.8	
	36	101.5	105.5	99.4	101.5	102.0	
Means		90.4	91.0	91.4	93.2		

Statistical differences			
Genotype	ns	Genotype x media (Figure 3)	ns
Media	ns	Media x embryo age (Figure 4)	**
Embryo age	***	Genotype x embryo age	**
Genotype x media x embryo age	**		

, Significant differences at level P ≤ 0.01; *, significant difference at level P ≤ 0.001; ns, not significant.

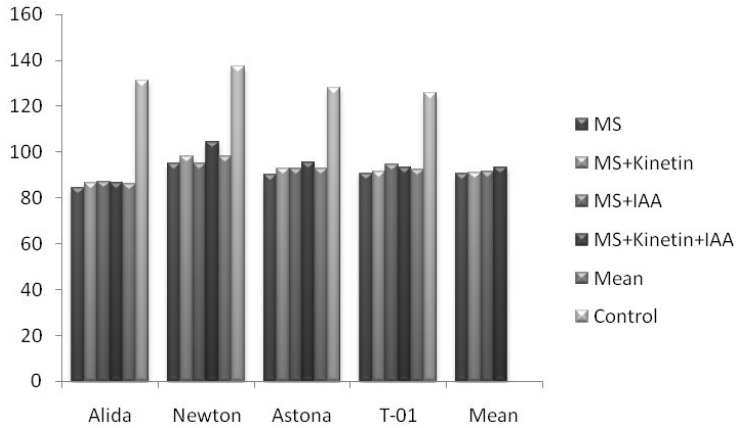

Figure 3. The time from pollination to flowering according to genotype.

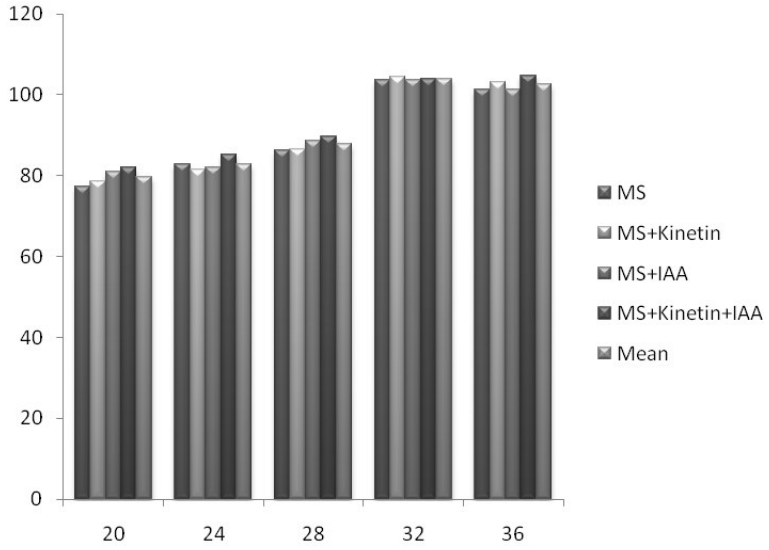

Figure 4. The time from pollination to flowering according to embryo age.

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