N.Z. Agronomy Society
Special Publication No. 5

Paper 68

EMBRYO RESCUE TECHNIQUES IN NECTARINE AND PEACH BREEDING

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ABSTRACT

The development of early-ripening peach and nectarine cultivars has been difficult because simple embryo rescue techniques have not existed. Embryo rescue was first used in the peach and nectarine breeding programme at Fresno in 1975. Procedures have been simplified and refined to permit growth of plants from embryos as small as 1 mm. As a result, embryos from genotypes ripening May 3-9 (58-64 days after bloom) have been grown and used as females in the breeding programme. The use of these earlier-ripening genotypes has advanced the maturity dates 10-30 days, depending on the parents used. A total of 39 000 embryos and 11 700 ovules have been cultured in our breeding programme (1975-1984). Such a large early-ripening population increases the chances of finding genotypes with desirable characteristics. To date, 355 seedlings have been selected of which 108 have been propagated for advanced testing or use as parents. Goldcrest peach and Mayfire nectarine are the result of our embryo rescue programme and were released in 1983. They are the earliest-ripening genotypes commercially available and the fruit has many desirable characteristics for the fresh market.

KEYWORDS

Early-ripening, *Prunus,* embryo culture, hybridsation.

INTRODUCTION

Embryo culture procedures for *Prunus* have been available since Tukey developed them in 1934. Blake was the first to use them in a peach breeding programme in 1937. Embryo culture methods are necessary to rescue embryos from early-ripening *Prunus* genotypes because the flesh ripens before the seed matures, and when the flesh starts to ripen, the seed aborts. Embryo rescue and culture allow the growth of seedlings from the hybridisation of early genotypes and the means of creating a population of very early-ripening genotypes. This provides a better chance of developing improved earlier-ripening cultivars.

The *Prunus* embryo culture programme at Fresno, California, was started in 1975 because the peach and nectarine industry needed earlier cultivars which have good commercial fruit characteristics. Many of the cultivars then available did not have all the desired commercial characteristics of size, shape, firmness, quality and colour. The earliest cultivars from which seed could be grown successfully in the breeding programme were from Flavorcrest peach which ripens June 20 and from Maygrand nectarine, which ripens June 15. These and later maturing peach and nectarine cultivars were hybridised with Springcrest, which ripens May 25; Springold, which ripens May 20; and Armking, which ripens June 5. The earliest ripening seedlings that existed in 1975 originated from these crosses. The earliest peach seedlings ripened May 20 to 25, no earlier than the earliest male parent used; and the earliest nectarine seedlings ripened June 10, slightly after the earliest male parent used. Therefore, it was obvious that to be able to develop cultivars earlier than material in the breeding programme, early-ripening cultivars needed to be used as female as well as male parents. It was not possible to do this in the past because the seed was not fully developed at the time the flesh was ripe and, therefore, could not be grown in soil.

MATERIALS AND METHODS

We started the embryo culture programme using the standard procedure and medium developed by Smith *et al.* (1969). Many of their procedures were laborious and the chemicals they used were toxic to people. Their procedure consisted of cubing the fruit so that no skin was left, surface sterilising the surface of the cubed fruit with phenol, cracking the pit open, surface sterilising the seed with merthiolate and rinsing three times in sterile distilled water. Thereafter, the embryo was removed from the seed and placed in the sterile medium. We developed a simpler and more effective method to prepare the seed for embryo culture. It simply consists of cracking open the fruit and pit, surface sterilising the seed by dipping it in 95% alcohol and then flaming it in a laminar-flow hood. It is important to point the micropyle end of the seed into the air flow to prevent the embryo from becoming too hot and being killed. When immature seeds were flamed for ovule culture, the integument was damaged and medium was not taken up. Surface sterilising the fruit with 70% alcohol for 30 seconds and 10% chlorox for 5 minutes and cracking open the fruit with double edge clippers was found satisfactory to obtain seeds that are free of contamination. If the pit is not

Table 1. The ripe dates of cultivars cultured and the earliest-maturing seedlings selected from their progeny.

Parent and year cultured		Date ripe Parent	Earliest-ripening selected seedling
FV9-164 OP[1]	1975	6/04/79	5/05/79
Springold OP	1976	5/21/79	5/10/79
Springcrest OP	1976	5/29/79	5/10/79
FV9-164 OP	1976	6/04/79	5/08/79
Armking OP	1976	6/07/79	5/14/79
Arm Queen OP	1976	6/10/79	5/21/79

[1]OP = open pollinated.

open to the atmosphere, the seed inside is clean. In one experiment more than 1000 seeds were cultured by this method without contamination.

The riper and juicier the fruit, the greater the chance of carrying contamination into the pit and ovule when it is cracked open. This problem can be reduced by picking the fruit at the firm-ripe stage.

RESULTS AND DISCUSSION

Embryos were grown successfully from Springold, Springcrest, FV9-164 (a sibling of Springold) and Armking, with the Smith, Bailey, and Hough (SBH) medium (Table 1). From these progeny we selected peach and nectarine seedlings that ripened as early as May 5 and May 14, respectively. The hybridisation of early genotypes and the rescue of these embryos by embryo culture made possible the development of even earlier-ripening genotypes. Table 2 shows the result of embryo culture on SBH medium for 1975 through 1979. Some cultivars had smaller embryos than those from Springcrest, Springold and Armking when they were ripe. To use these genotypes as females, media improvements needed to be made. The Murashige and Skoog (MS) (1962) medium with potassium succinate, L-glutamine, and 3% sucrose was more suitable for growing these small embryos than SBH medium. Embryos as small as 5 mm could be grown with limited success on MS medium. We first used this procedure in the breeding programme in 1980.

Some of the selections made from embryo cultured material that ripened on May 10 to 15 have embryos that are at most 1 mm long or less. The use of these selections as female parents again required improved media and techniques. In 1978 we started experiments based on the cotton ovule culture procedure (SH) of Stewart and Hsu (1977) and thus developed a similar procedure for *Prunus* (Ramming, 1985). This procedure allows the embryo to increase in size and dry weight within the seed by using the nucellus and endosperm and the supplementary nutrients from the artificial medium on which they are plated. The other advantage is the action of the seed coat acting as a natural inhibitor of germination which prevents precocious germination. Precocious germination is a problem in embryo culture when embryos are held at room temperature for development.

Our system is based on maximum support of seed development followed by stratification and direct planting

Table 2. The number of ovules and embryos cultured, % seedlings planted in the greenhouse and field, % contaminated, % small embryos that did not grow, and number of seedlings selected and propagated in the peach and nectarine breeding programme for 1975-84.

Year	Number ovules	Number embryos	To greenhouse		Percent To field	Contaminated	Small	Number Selected	Propagated
1975	—	1075	3		3	42	55	11	7
1976	—	2571	58		45	1	29	102	24
1977	—	2066	56		40	4	33	47	14
1978	—	4214	79		72	12	8	53	23
1979	—	6277	67		54	15	14	54	15
1980[1]	—	8129	54		32	22	9	49	11
1981[2]	1419	6058	58		30	8	32	25	10
1982[3]	2140	5420	52		10	20	28	14	4
1983	5080	1281	41	(34 OC[4] 70 EC[5])	26	11	35	—[6]	—
1984	3072	2102	38	(20 OC 64 EC)	33	15	24	—	—

[1]MS as well as SBH media used and seed sterlised by flaming, starting year indicated.
[2]Ovules cultured on cotton support in petri dish. Planted ovules as seed in greenhouse only year indicated.
[3]Ovules cultures on SH media and sub-cultured in test tubes as embryos starting year indicated.
[4]OC = Ovule culture.
[5]EC = Embryo culture.
[6]Seedlings not fruiting.

Table 3. The number of ovules and embryos cultured in 1983 and 1984 and the percent planted in the greenhouse.

	Ovule culture		Embryo sub-cultured from ovule culture				Embryo culture			
Year	Number	Percent viable embryos	Number on MS[1]	Percent planted	Number on SBH[1]	Percent planted	Number on MS[1]	Percent planted	Number on SBH[1]	Percent planted
1983	5080	70	2325	35	1240	73	226	6	1055	84
1984	3072	50	713	26	839	78	360	35	1753	86

[1]The determining factor if embryos were cultured on MS or SBH media was embryo length. Embryos less than 10 mm were cultured on MS and those 10 mm or greater were cultured on SBH medium.

of seed into the soil. This system was used in 1981, the first year ovule culture was used in the breeding programme. However, we found the percent germination could be increased by extracting the embryos from the ovules after a two-week culture period at 80 °C, then placing the embryos on solid medium, stratifying the embryos, and germinating them in the same nutrient medium. This procedure was used in 1983 and 1984 to grow seedlings from genotypes that mature May 3 to May 9 (Table 3).

Many selections have been made and saved from among seedlings that resulted from the embryo rescue programme (Table 2). Almost 3% of the seedlings grown have been selected for at least a second evaluation. Therefore, this technique has been very useful for the development of early-ripening germplasm. As a result of the embryo rescue programme one early peach, Goldcrest and one early nectarine, Mayfire, were introduced. Goldcrest is the earliest-ripening peach cultivar commercially grown in California that has good size, shape, firmness, and colour relative to other cultivars that currently exist. Mayfire ripens earlier than the earliest commercial cultivar, Maybelle, a mutation of Armking. Currently, Mayfire fills a need for an early-ripening cultivar that has high colour, desirable firmness, and good quality.

Aspects important to the success of the embryo culture programme include the awareness and use of proper sterile procedures and an adequate means of plant acclimatisation. As shown in Table 1, the percent contamination was very high in 1975 (40%). This reflects the inexperienced tissue culture technical help. In 1976, an experienced technician specifically trained in plant tissue culture, reduced embryo contamination to less than 2%. The humidification system should provide high humidity without the accumulation of free water on the plants, a condition that is optimal for acclimatising the plants moved from the test tube into soil of the greenhouse. It is also important to have a skilled technician who can perceive problems in the greenhouse culture and correct them before they become damaging.

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SYMPOSIUM DISCUSSION

Dr M.J. Carson, Forest Research Institute

Have you had any evidence of any genetic change associated with culture techniques?

Ramming

Let me first say that generally our objective is to produce one plant per embryo and we are trying to create a population by sexual hybridisation, so essentially we have not seen that. However recently, in an attempt to get better shoot formation from some of our seedless grape embryos, we put some 2-4D in the media. We got a number of somatic embryos formed. We do not know yet if they are variable or not — we are just in the beginning stages.

Carson

Is there any indication of a correlative response in the traits that you are interested in? For example in selecting for early ripenining are you finding that other traits are changing in their frequency?

Ramming

We are really using the technique to provide a method whereby we can grow the seed from the early ripening genotypes. In our crossing plan we also cross genotypes that have the desirable traits that we are trying to incorporate into one individual. Therefore, for example, we might have a large-fruited early ripening peach, and we are crossing it with a high colour early-ripening peach, trying to pull in these

other traits as well as the earliness.

Dr R.D. Burdon, Forest Research Institute

Has anybody tried the approach of delaying fruit ripening by the application of cytokynin in order to allow embryos to mature more or less naturally.

Ramming

Yes, there has been a little bit of work done on this in Florida. They were able to hold the fruit about a week on the tree but I do not recall the additional size or the additional percentage of survival from those embryos being measured. There has also been the thought that if the temperature at which the plant is growing was reduced maybe it would take longer for the fruit to mature and thereby allow the embryo a better chance to develop. However, that has actually operated in the reverse because it has also retarded embryo development in these cooler temperatures.

Burdon

That is very similar to what we have observed with conifers — often in seed orchards you find embryo development is very poor inside the seeds in cool environments.