Article Id
AL04518

GERMINATING UNDER-DEVELOPED SEEDS OF KINNOW MANDARIN AND MOSAMBI ORANGE FOR IDENTIFICATION OF TRIPLOID SEEDLINGS

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Triploid breeding in citrus is widely employed for the development of seedless cultivars; however, recovery of triploid progeny is often constrained by poor seed development and low germination rates, particularly in tetraploid x diploid crosses. The development of seedless cultivars is a major breeding objective driven by consumer preference and market demand (Kiran *et al*., 2024a, b). Triploid breeding has emerged as an effective approach to achieve seedlessness in citrus (Aleza *et al*., 2010). Triploids are generally obtained through crosses between tetraploid and diploid parents. However, such crosses often result in under-developed or abortive seeds due to endosperm imbalance and embryo abortion (Esen and Soost, 1973). These seeds typically fail to germinate under natural conditions, limiting the recovery of triploid progeny. Conventional embryo rescue techniques using *in vitro* culture are effective but expensive and technically demanding. The citrus triploids are generally developed by crossing tetraploids with diploids, but the resultant seeds do not germinate under normal conditions because of poor seed development and to germinate them, *in vitro* tissue culture seedling raising protocols are followed, which are very tedious and costly too.

A protocol for germination of less-developed seeds under normal conditions in the laboratory was tried by sowing them in petri-plates (Fig. 1). The sowing of poorly developed and bold seeds of tetraploids of Kinnow and Mosambi was done for the identification of triploids with the help of chromosome counting in the root tips and by flow cytometry. The seeds were washed properly and put in moist tissue paper. Both the seed coats were removed without disturbing the embryos and cotyledons. The seeds without seed coat were put on moist tissue paper laid in the petri plates. The tissue papers were kept moist continuously by adding a few drops of water in the petri dishes as and when required. After about 10 days, gemmation commenced and the resultant seedlings were transplanted in the seed trays filled with commercial soil mixture (soil: sand: FYM in 1:1:1 ratio) after one month of seed sowing (Fig.

2 and 3). The seedlings were maintained in seed trays under laboratory conditions for about four months and then shifted to the field in the raised beds. In total, 11 seedlings from poorly developed seeds of Kinnow mandarin and 91 seedlings from Mosambi were transplanted in the field for detailed evaluation and for confirmation of triploidy using morphological, physiological, cytological and flow cytometrical techniques (Fig. 4). On transplanting to field, all the seedlings survived and no mortality was recorded as generally observed in hardening process in *in vitro* raised plants (Table 1).

This technique of germinating under-developed seeds under *in vivo* conditions can prove useful as it does not require any *in vitro* conditions, which are comparatively very costly in nature. The technique is also user-friendly and can also be tried for fast germination of the normal seeds in the nursery and can save lot of resources. Further, the protocol eliminates the need for *in vitro* embryo rescue and provides a practical alternative for citrus breeding programs.

Table. 1. Seeds sown, germinated and successful transfer of seedlings in nursery beds

Genotype	Seed sown		Seeds germinated		Germination (%)		Transplanting of seedlings in raised beds	
2021	Under-developed	Bold	Aborted	Bold	Aborted	Bold	Transplanted	Survived
Mosambi	120	165	25	94	20.83	56.97	14	14
Kinnow mandarin	75	105	5	32	6.67	30.48	12	12
2022								
Mosambi	320	720	77	504	18	70	77	77
Kinnow mandarin	495	860	89	594	24	69	11	11

Fig. 1. Under-developed and bold seeds

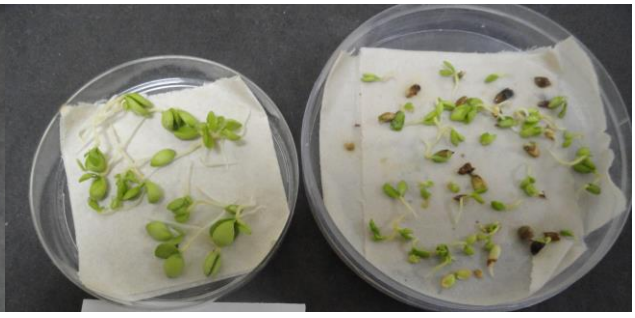

Fig. 2. Germination in bold & under-developed seeds

Fig. 3. Transferring germinated seeds in the plastic trays carrying commercial soil mixture

Fig. 4. Seedlings from under-developed seeds established in the field beds.

Conclusion

A simple, efficient, and low-cost *in vivo* germination protocol was standardized for under-developed seeds of Kinnow mandarin and Mosambi orange. The method successfully enabled recovery of viable seedlings suitable for triploid identification without the need for *in vitro* embryo rescue. This approach can significantly benefit citrus breeding programs, especially in resource-limited settings, and may be applicable to other crops exhibiting seed abortion problems.

References

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