Pak. J. Bot., 35(4): 557-561, 2003.

TOWARDS A SEEDLESS CULTIVAR OF KINNOW MANDARIN V. EMBRYOGENESIS OF PICKED AND DROPPED FRUITS

NAFEES ALTAF AND M. MOHSIN IQBAL

Nuclear Institute for Agriculture and Biology (NIAB), P. O. Box 128, Faisalabad, Pakistan.

Abstract

Nucellar polyembryony in picked and dropped fruits was studied. There were 2,3, 4 and 5 leaf embryos with balanced normal germination. The significant differences were imbalanced growth with 8.7% thin shrinked leaves in embryos of picked fruits and 12.9% embryos without root system in dropped fruits which upon grafting had 100% plants with leaf abscission trait. Presence of abscission trait in vegetative parts of the branch and in the embryo indicated that the trait is of genetic origin as the embryos are derived from cell lines present in the vegetative parts.

Introduction

Citrus has a top position in fruits production and Kinnow mandarin is the dominant commercial cultivar because of high yield due to its suitability to soil and environmental conditions of the Punjab, Pakistan. No other country in the world can match the production of quality Kinnow fruit with us. The only drawback is the lack of uniformity in fruit characteristics including its seedy nature. The broad objective of the study is the improvement of Kinnow mandarin which is a continuous effort within a limited gene pool of the cultivar with the specific objective to develop seedless with good quality fruit and fruit holding potential to tree. Some yield losses are due to stress-induced fruit drop. Fruit and leaf drop is due to abscission caused by abscisic acid (ABA) which is a naturally occurring plant growth regulator implicated in the control of a wide range of essential physiological processes including plant development and plant adaptation to environmental stress (Giraudat *et al.*, 1994). Cloning of seedless trait with no-drop fruit character by embryo/shoot apex/sprout grafts in contrast to conventional method of bud wood grafting which seems to be a possible path for isolation of new variants from existing natural somatic variability since above mentioned grafts fixes the characters of any somatic cell line immediately. Mendel (1981) studied *Citrus* varieties originated at a rate of about 15 to 20% as bud mutation. The number of inferior bud mutation is much larger than that of desirable types because most of the mutations are useless. Somatic mutations that have occurred in Texas in the past 60 years (Richard & Hensz, 1981) have produced the cultivars that constitute nearly 100% of the grape fruit and 50% of the orange plantings. This work will help in making seedless/ low seed Kinnow clones with fruit holding potential to tree.

Materials and Methods

The following types of low seeded fruits were used in the study:

1. Picked fruits with strong holding to tree, were harvested from the orchard.
2. Dropped fruits which already dropped because they were extremely loosely connected to tree and were with yellow ring at the stalk attachment part.

The fruits were sterilized and dissected, the seeds were taken out and both testa of the seeds were removed. Embryo were cultured according to the procedure mentioned by Altaf *et al.*, (2002). The data was recorded after 35 days of embryo culture (Table 1).

Different embryo types were top-grafted onto rootstock seedlings. Sprouts from the low seeded branches from where the picked and dropped fruits were taken were also grafted onto rootstock seedlings to find any differences in the 2 types of sprout grafts.

Results

In picked fruits their were one embryo type of 5 leaf; embryo with large leaf and 2 very thin leaves; one small leaf and other leaf with 2 mid-rib. Thin shrinked leaves (one, two, three and four) were 8.7% in picked fruits only. The maximum frequency was of 2 leaf (27.6%) and (34.7%) in picked and dropped fruits. Three leaf embryos were 17.8% and 7.4% in picked and dropped fruits respectively. Four leaf embryos were 2.2% in picked and 0.4% in dropped fruits (Table 1).

Embryos with very thin leaves (4.9%) were present in dropped fruits only. Embryos with elongated roots were 5% and 3.2% in picked and dropped fruits respectively. Embryos without developed root system were (1.3%) in picked and (12.9%) in dropped fruits. If such embryos were grafted on rootstock seedling, they dropped leaves in harsh temperatures with a cycle of new emergence of leaves and drop. The dropped fruits have some seeds of broader micropylar end (Altaf *et al.*, 2003) irrespective of the shape and size of seed.

Table 1. Embryogenesis from picked and dropped fruits.

	Picked Fruits		Dropped Fruits	
Embryo types:	**No.**	**%**	**No.**	**%**
1. 5 Leaf	1	(0.185)		
2. 4 Leaf	12	(2.222)	2	(0.352)
3. One of the 4 leaf with 2 mid-rib	1	(0.185)	0.00	(0.00)
4. 3 Leaf	96	(17.777)	42	(7.394)
5. 2 Leaf	149	(27.593)	197	(34.683)
6. 1 Leaf	22	(4.074)	33	(5.810)
7. Weak embryos + Distorted shapes	55	(10.185)	99	(17.429)
8. Meristemless	47	(8.704)	30	(5.282)
9. Elongated roots	27	(5.000)	18	(3.169)
10. 1 small +1large leaf	25	(4.630)	10	(1.760)
11. Cot. + root	41	(7.593)	30	(5.282)
12. Thin shrinked (1,2,3,4)leaves	47	(8.704)		
13. 1 leaf with 2 mid-rib	2	(0.370)	1	(0.176)
14. No germination	6	(1.111)	5	(0.880)
15. 1 large+2V.thin leaves	1	(0.185)		
16. 1 small leaf + 1 leaf with 2 mid-rib	1	(0.185)		
17. Embryos without developed root system	7	(1.296)	73	(12.852)
18. V. thin leaves			28	(4.929)
Total embryos:	**540**		**568**	

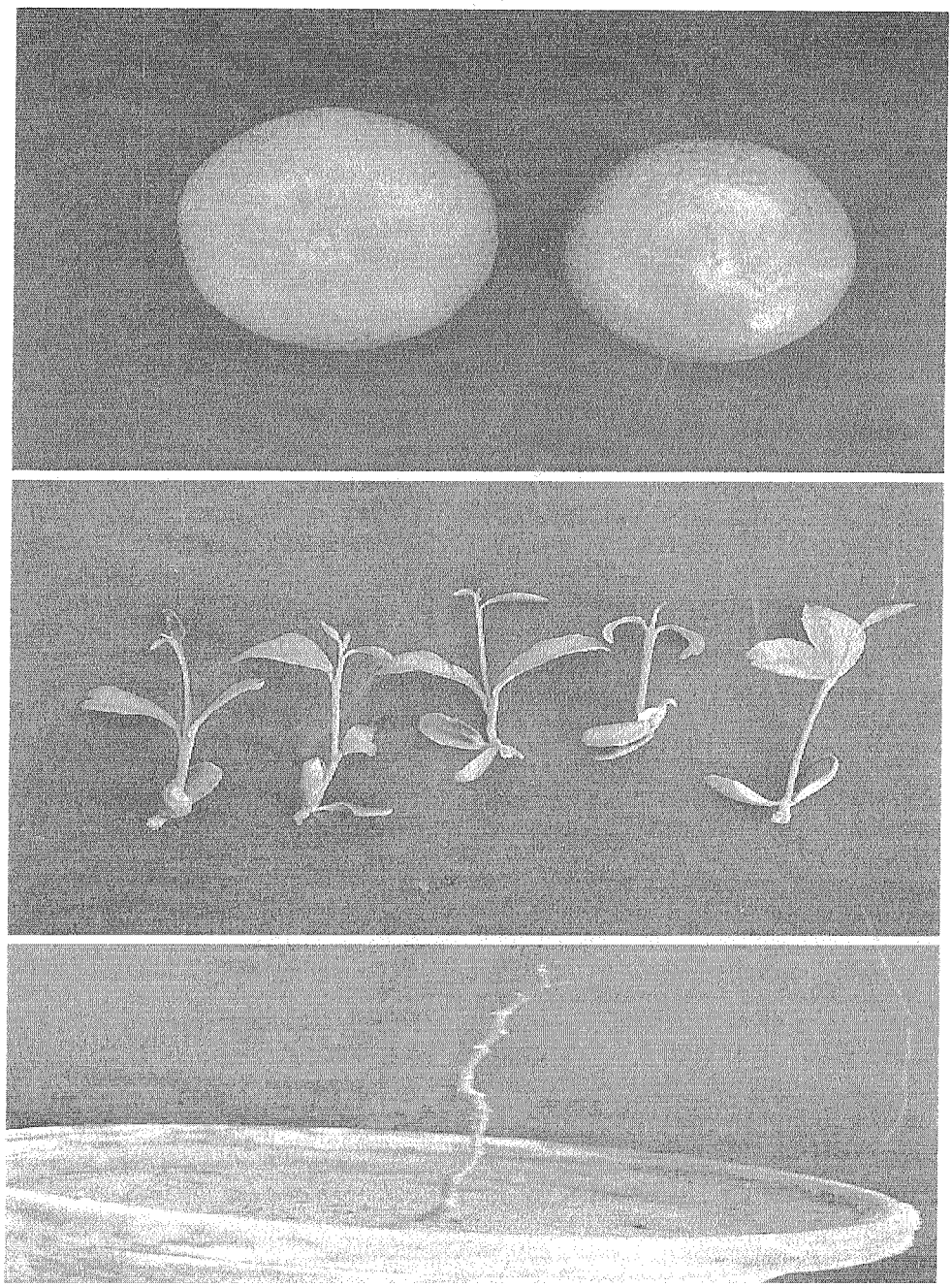

Fig. 1.
Upper – dropped fruits with yellow ring at the peduncle attachment part.
Middle – embryos with no root system.
Lower – grafted embryo with leaf abscission trait.

Sprouts from the branches/trees which have fruit drop and which have strong holding potential of fruit to tree were grafted. The former sprouts had a tendency of leaf drop upon grafting within one week, while the latter had holding of leaves on sprouts stem. This clearly indicated that there is leaf fall tendency in sprouts of branches which have fruit drop character upon grafting which is a stress condition. Usually there is June and October drop in Kinnow. A large number of flowers are formed. Finally a small fraction of fruits stay till harvesting period in Kinnow where leaf, flower and fruit drop is normal, however, there are differences in intensity of abscission in various trees/branches. Trees/branches with dense foliage usually have potential to hold fruits in Kinnow. The tree which have fruit drop problem are sensitive to harsh climatic conditions such as extreme low temperature (less than 2°C) and fog or mist in cold days. High temperature (above 45°C) favour fruit drop in Kinnow. Table1 and Figure 1 gives clear link between embryo and abscission trait.

Discussion

Kinnow mandarin growing commercially in Punjab is one of the best example of successful introduction. Natural variability existing in commercial cultivar and identification of superior types with respect to yield, fruit quality including seedlessness, bearing period with no or minimum fruit drop and resistance against biotic stresses are important. So selection of superior clones in commercial dominant large scale propagated cultivar is essential. Kinnow mandarin has 75% young fruit drop (Chaudhry, 1994) and upto 30% full grown fruit drop which is also in low seeded fruits. It was suggested that in *Citrus*, removal of either old or young leaves advanced and increased abscission and reduced the sucrose/hexose ratio of fruit set (Mehouachi *et al.*, 2000). The growth characteristics as the top-root ratio and ABA level in the *Citrus* rootstock seedlings seemed to affect the performance of grafted trees (Noda et al., 2001). In plants, biological constraints to seed and fruit production, beside other factors, are floral, ovule and embryo abortion, failure of seeds and fruits to mature (Owens, 1995). The concentration of endogenous IAA and ABA, the ratio of ABA/IAA and the level of putrescine and spermidine were related to ovule abortion and nucellar embryo initiation and development in *Citrus tankan*, especially the balance of ABA and IAA was critical to regulate development of ovules and embryos (BiGuany *et al.*, 1997).

Natural superior mutations in *Citrus* cultivars are important source of new germplasm which may appear as bud sport (Long, 2000) or as seedling selection (Muller, 1985). The useful genetic variability in Ponkan mandarin accessions has been studied by Coletta-Filho *et al.*, (2000). Superior clonal selections have been made in Nagpur mandarin by Singh & Singh, (2001). Ricalate which is a spontaneous bud mutation of Washington naval orange with a delay of fruit coloration, high fruit firmness and high attachment of mature fruits to the peduncle has been studied by Zacarias *et al.*, (1993). The altered abscission in Ricalate either in fruits on leaves was more obvious with the development of the tissue, being highly resistant to abscission at later stages of development. The present study confirmed that high attachment of fruit to peduncle can be selected from genetically variable population.

The result of the present study indicated differences in embryogenesis of picked and dropped fruits of Kinnow mandarin preferably from low seeded branches. The embryos without developed root system are more in full grown dropped fruits which have yellow ring at the peduncle, moreover these embryos upon grafting on rootstock seedlings had 100% leaf abscission. The link between above type of embryo with leaf abscission is a

clear indicator of getting rid of abscission trait in embryo derived plants. The balanced germinated embryo growth depends very much on its own structure as well as on the rootstock seedling. However the embryos derived from low seeded fruits are sensitive to extreme environment and require extra care for atleast one year to support their growth.

Acknowledgements

Scientific assistance of Mr. Anees Ahmad, Mr. Liaquat Ali, Mr.Inkisar Ahmad is gratefully acknowledged. The work was partially sponsored by PARC, ALP Project identification No. 01-02-01-10.

References

Altaf, N., M.M. Iqbal, E.K. Murwat, A. Gulnaz, A. Sattar, G.A. Chaudhry, I.A. Hafiz and M. Ashraf. 2002. Towards a seedless cultivar of Kinnow mandarin. 1. Embryogenesis of low seeded/seeded fruits. *J. Agric. Res.*, 40(1): 29–35.

Altaf, N., M. Mohsin Iqbal, A. Gulnaz and E.K. Marwat. 2003. Towards a seedless cultivar of Kinnow Mandarin. II. Variation in seed shape and seed size. *Pak. J. Bot.*, 35(1): 79–87.

BiGuang, H., Ye-MingZhi and Lu-Liuxin. 1997. The dynamics of endogenous growth regulators and polyamines in young fruits of Jiaogan and their relationships to seed abortion. *J. of Fujian Agricultural Univ.*, 26(4): 411–415.

Chaudhry, M.I. 1994. Fruit crops. In: *Horticulture*" (Eds.): E. Bashir and R. Bantel, National Book Foundation, p. 449.

Coletta-Filho-HD, M.A. Machado, M.L.P.N. Targon and J. Pompeu. 2000. The use of Random Amplified Polymorphic DNA to evaluate the genetic variability of Ponkan mandarin (*Citrus reticulata* Blanco) accessions. *Genetics and Mol. Biol.* 23:(1): 169–172.

Giraudat, J., F. Parcy, N. Bertanche, F. Gosti, J. Leung, P.C. Morris, M.B. Durandand and N. Vartarian. 1994. Current advances in abscisic acid action and signalling. *Plant Molecular Biology* 26: 1557– 1577.

Long, Y.Y. 2000. "Longyan Hongyou", a sport of Guanximiyou pummelo variety. *South China Fruits*, 29(2): 6.

Mehouachi, J., D.J. Iglesias, F.R. Tadeo, M. Agusti, E. PrimoMillo and M. Talon. 2000. The role of leaves in *Citrus* fruitlet abscission: Effects ion endogenous gibberellin levels and carbohydrate content. *J. Hort. Sci. Biotech.*, 75(1): 79–85.

Mendel, K. 1981. Bud mutation in *Citrus* and their potential commercial value. *Proc. Int. Soc. Citriculture*, 86–89.

Muller, P. 1985. A promising new red pummelo. *Information Bulletin, Citrus and Subtropical Fruit Research Institute*, 154(2): p. 4.

Noda, K., H. Okuda and I. Iwagaki. 2001. Relationship between growth and IAA and ABA levels in *Citrus* rootstock seedlings. *J. Japanese Soc. Hort. Science*, 70(2): 258–260.

Owens-Jn. 1995. Constraints to seed production: temperature and forest trees. *Tree Physiology*, 15(7–8): 477–484.

Richard, A. and Hensz. 1981. Bud mutation in *Citrus* cultivar in Texas. *Proc. Int. Soc. Citriculture*, 89–91.

Singh, A. and S. Singh. 2001. Effect of lanking of branches on the quality of Nagpur mandarin fruits. Annals of Biology, 17(1): 71–74.

Zacarias, L., F.R. Tadeo, R. Bono and E. Primo Millo. 1993. Abscission studies in a new mutant of navel oranges. *Proc. Int. Symp. On Cellular and Molecular Aspect. Biosynthesis and Action of the Plant Hormone Ethylene*, Agen, France, Aug. 31–Sept. 4, 1992. Dordrecht, Netherlands; Kluwer Academic Publishers (1993) 284–290 ISBN 0-7923-2169-3.

(Received for publication 12 May 2003)