

USING TISSUE CULTURE FOR VEGETATIVELY PROPAGATING AND IMPROVING ASPARAGUS PRODUCTION AND QUALITY

W.J. CLORE and HSU-JEN YANG

Washington State University
Irrigated Agriculture Research and Extension Center
Prosser, Washington 99350

Abstract. Tissue culture of asparagus provides a technique for mass production of superior clonal material for increasing production by mass production of staminate clones; increasing proven parents that result in superior crosses for the production of large quantities of high quality F₁ seed; and for developing pathogen-free stock.

REVIEW OF LITERATURE

Asparagus officinalis L. is a dioecious crop of pistillate and staminate plants occurring in equal numbers when grown from seed. Because of the heterogeneous nature of asparagus, propagation by seed results in individual plants of varying yielding ability (3, 4, 7, 10, 11, 18, 28). By establishing a field of a superior selection of genetically-identical staminate plants yields should be markedly increased (11, 18). We have found the growth of such plants to be very uniform and a tendency for new spears to emerge together after removal of all above-ground asparagus. Such a development would promote mechanical harvesting of this crop if mass production of clonal material becomes commercial feasible.

Prior to the development of the asparagus tissue culture (TC) techniques vegetative propagation by crown division only resulted in a few additional plants. The propagation of asparagus by TC not only makes it possible to propagate genetically-identical plants for production, but provides a means of increasing parents of superior crosses for commercial seed production.

Propagation of asparagus plants by callus and cell culture has been demonstrated (12, 19, 20, 21, 23) but some tetraploid and aneuploid cells have resulted. It has been shown that normal diploid plants can be developed from stem tips (1, 5, 6), meristem (2, 15), shoot apexes (8, 16), and stem lateral buds (13, 14, 24, 25, 27). Meristem and shoot apex cultures have proven to be better methods for developing normal plants, but require rather delicate techniques in excising the minute apical meristem of the shoot apex. Much time is required and the survival of propagants is poor. However, this technique does provide a method of developing disease-free plants of asparagus and other horticultural crops (9).

METHODS AND RESULTS

We have developed a more simple method of vegetative propagation that avoids the problems involved in callus formation (24, 25, 27). By using the TC technique aseptic stock plants are established from lateral buds of asparagus spears. From these stock plants buds are taken and developed into rooted plantlets under sterile conditions.

After these complete plantlets are developed they are removed from the sterile environment and placed under mist in Jiffy-7 peat pots (26). When shoots start to grow they are transplanted into pots containing soil. This is followed by maintaining these growing plants in the greenhouse for 3 to 4 months until they become well developed. They are then acclimated in a 50% shade lathhouse 1 to 2 weeks before setting into the field. Early spring or late fall planting has resulted in the best crown survival.

The nutrient medium and culture environment for TC techniques have been described by Murashige (16) and modified by Yang and Clore (24, 25, 27). A plentiful supply of aseptic stock plants is necessary for mass clonal multiplication. To do this it is important to consider stem vigor, bud age, bud position, implantation techniques, and concentration of plant growth substances.

The propagation time table using stock plants for mass production of asparagus crowns is as follows:

- a) 6 to 8 months to develop stock plants from asparagus spear buds;
- b) 2½ months to develop complete plantlets from bud cultures;
- c) 1 month to develop complete plantlets from non-rooted plantlets;
- d) 1-2 weeks in mist chamber after transplanting from sterile culture to Jiffy-7 peat pots;
- e) 3-4 months in greenhouse to develop plant size for field planting;
- f) 1-2 weeks acclimation in lathhouse before transplanting to the field.

Thus the total time required from spear bud to field planting of large numbers of clonal asparagus is 13 to 16 months. It has been estimated that with an adequate supply of aseptic stock plants and adequate facilities one person working 200 days in one year should be able to produce approximately 70,000 plants.

LITERATURE CITED

1. Andreassen, D.C. and J.H. Ellison. 1967. Root initiation of stem tip cuttings from mature asparagus plants. *Proc. Amer. Soc. Hort. Sci.* 90:158-162.

2. Bourgin, J.P., J.F. Muller, and G. Morel. 1973. La multiplication vegetative de l'Asperge (*Asparagus officinalis* L.). 96^e Congrès national des sociétés, Toulouse, 1971. *Science*, t. IV:107-112.
3. Currence, T.M. and A.L. Richardson. 1938. Asparagus breeding studies. *Proc. Amer. Soc. Hort. Sci.* 35:554-547.
4. Ellison, J.H. and D. Scheer. 1959. Yield related to brush vigor in asparagus. *Proc. Amer. Soc. Hort. Sci.* 73:339-344.
5. Galston, A.W. 1948. On the physiology of root initiation in excised asparagus stem tips. *Amer. J. Bot.* 35:281-287.
6. Gorter, C.J. 1965. Vegetative propagation of *Asparagus officinalis* by cuttings. *J. Hort. Sci.* 40:177-179.
7. Hanna, G.C. 1942. Correlation studies of asparagus comparing yields of various shorter periods with ten-year yields. *Proc. Amer. Soc. Hort. Sci.* 41:321-323.
8. Hasegawa, P.M., T. Murashige, and F.H. Takatori. 1973. Propagation of asparagus through shoot apex culture. II. Light and temperature requirements, transplantability of plants, and cytological characteristics. *J. Amer. Soc. Hort. Sci.* 98:143-148.
9. Ingram, D.S. 1973. Growth of plant parasites in tissue culture pp. 393-421. In *plant tissue and cell culture*. H.E. Street (ed.). Univ. Calif. Press., Berkeley, Calif.
10. Ito, P.J. and T.M. Currence. 1965. Inbreeding and heterosis in asparagus. *Proc. Amer. Soc. Hort. Sci.* 86:338-346.
11. Jones, H.A. and W.W. Robbin. 1928. The asparagus industry in California. *Univ. Calif. Agr. Exp. Sta. Bul.* 446.
12. Malnassy, P. and J.H. Ellison. 1970. Asparagus tetraploids from callus tissue. *HortScience*. 5:444-445.
13. Matsubara, S. and W.J. Clore. 1973. Vegetative propagation of asparagus from lateral buds. *Sci. Rep. Fac. Agr. Okayama Univ. Jap.* 43:19-26.
14. _____. 1973. Population effect in lateral bud culture of asparagus and promotion of root formation by transplanting. *J. Jap. Soc. Hort. Sci.* 42:142-146.
15. Muller, J.F., J.P. Bourgin, and G. Morel. 1973. La culture *in vitro* du meristem caulinaire de l'Asperge. *Eucarpia 4^{eme} reunion sur la selection de l'Asperge*. Versailles, France. 1973. p. 133-143.
16. Murashige, T., M.N. Shabde, P.M. Hasegawa, F.H. Takatori, and J.B. Jones. 1972. Propagation of asparagus through shoot apex culture. I. Nutrient medium for formation of plantlets. *J. Amer. Soc. Hort. Sci.* 97:158-161.
17. _____. 1974. Plant propagation through tissue cultures. *Rev. Plant Physiol.* 25:135-166.
18. Robbins, W.W. and H.A. Jones. 1925. Secondary sex characters in *Asparagus officinalis* L. *Hilgardia* 1(9):183-202.
19. Steward, F.C. and M.O. Mapes. 1971. Morphogenesis and plant propagation in aseptic cultures of asparagus.
20. Takatori, F.H., T. Murashige, and J.I. Stillman. 1968. Vegetative propagation of asparagus through tissue culture. *HortScience* 3:20-22.
21. Wilmar, C. and M. Hellendoorn. 1968. Growth and morphogenesis of asparagus cells cultured *in vitro*. *Nature* 217:369-370.
22. Yakuwa, T., T. Harada, K. Saga, and Y. Shiga. 1971b. Studies on the morphogenesis of asparagus. II. Effect of auxin and 6-benzyladenine on callus and organ formation of stem pieces cultured *in vitro*. *J. Jap. Soc. Hort. Sci.* 40:347-353.
23. _____, _____, N. Inagaki, and Y. Shiga. 1972. Studies on anther culture of

- horticulture crops. I. Induction of callus and differentiation of organs in anther culture of asparagus. *J.Jap. Soc. Hort. Sci.* 41:272-280.
24. Yang, Hsu-Jen and W.J. Clore. 1973. Rapid vegetative propagaion of asparagus through lateral bud culture. *HortScience* 8:141-143.
25. _____ and _____. 1974a. Development of complete plantlets from moderately vigorous asparagus shoots of stock plants *in vitro*. *HortScience* 9:138-140.
26. _____ and _____. 1974b. Improving the survival of aseptically-cultured asparagus plants in transplanting. *HortScience* 9:235-236.
27. _____ and _____. 1974. *In vitro* reproductiveness of asparagus stem segments with branch-shoots at a node. *HortScience*. 10:411-412.
28. Young, R.D. 1939. Yield-growth relationships in asparagus *Proc. Amer. Soc. Hort. Sci.* 35:576-577.

MASS PRODUCTION OF BOSTON FERN THROUGH TISSUE CULTURE

RANDALL W. BURR

Transplant Nursery, Inc.
Oxnard, California

I will discuss the practical aspects of tissue culturing for the purpose of mass-producing plants for a commercial nursery. It has just been in the last few years that the work of tissue-culturists, such as Dr. Tosh Murashige at the University of California, Riverside, has been tried in practical applications by commercial nurseries. This discussion will concern the application of tissue culture for the mass production of Boston fern [*Nephrolepis exaltata* 'Bostoniensis']. We have learned much from Dr. Tosh Murashige at the University level, but we have found that their procedures and techniques must be amended when applying them to a commercial lab. We haven't the funding, time, nor the high quality of personnel that a University has, so we have adopted their techniques to our own special requirements.

The actual proliferation of the ferns begins with the stolon tips, or runner tips, from a parent plant. The tips are collected and brought to the "clean air" station where they are sterilized by placing them in a bleach solution for a given amount of time, then they are run through sterile water baths to assure the removal of any bleach residue. At this time they are then placed on the medium which is enclosed in a culture tube or flask. From here they go to the culture room for growing. The initial growing time from this tip to plants large enough to divide takes about 2 to 3 months. After this initial 3 months the plants are divided into individual plants and placed back on fresh medium. It now only