IN VITRO PROPAGATION OF 'CHANDLER', 'VINA' AND 'SUNLAND'

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ABSTRACT

Walnut varieties are typically propagated by grafting onto seedling rootstock. Little research has been done on varieties grown on their own roots because of difficulties in propagation. Conventional means of propagation have been unsuccessful and mature walnut varieties have been described as recalcitrant in vitro. In vitro propagation of mature varieties of Persian walnut could result in major economic benefits for the walnut industry. If commercial varieties perform satisfactorily on their own roots, the expensive time-consuming process of grafting could be avoided, genetic uniformity could be assured and trees would not be subject to the lethal girdling of blackline disease. In addition, mature clones on their own roots might be more precocious than grafted seedlings. These factors are becoming increasingly important as more walnut growers are planting high density or hedgerow orchards. Through work described in this report 3 walnut varieties have been multiplied in vitro and planted in the field for further evaluation. This success was achieved through modifications in handling procedures rather than in medium formulation. Modifications in standard procedure included a 3 hr running water wash prior to sterilization, basal trimming, and rapid transfer.

OBJECTIVES

In vitro propagation of mature varieties of J. regia L. (Persian or English walnut) could result in major economic benefits for the walnut industry. If commercial varieties perform satisfactorily on their own roots, the expensive and time-consuming process of grafting could be avoided, genetic uniformity could be assured and trees would not be subject to the lethal girdling of blackline disease. In addition, mature clones on their own roots might be more precocious than grafted seedlings. These factors are becoming increasingly important as more walnut growers are planting high density or hedgerow orchards.

Methods for in vitro propagation of Paradox walnut and northern California black walnut (J. hindsii) have been published recently. The same methods were found to be satisfactory for juvenile seedlings of J. regia but mature J. regia cultivars were reported to be recalcitrant in culture and susceptible to latent contamination and slow decline within a few months of being initiated into culture.

The goal of this study was to obtain commercial cultivars of J. regia on their own roots. We report here the methodology that we used to successfully micropropagate J. regia cultivars 'Sunland' and 'Vina'. Methodology was developed using 'Chandler' in preliminary studies.

PROCEDURES

'Chandler' material was initiated into culture in June 1985 from 15 year old trees in the University of California, Davis variety collection.
'Sunland' and 'Vina' were initiated in mid-October 1985 from 18 month old grafted trees which had been sprayed weekly with a solution of benzyladenine (BA) (100 mg*liter\(^{-1}\)) and gibberellic acid (GA\(_3\)) (50 mg*liter\(^{-1}\)) to induce vigorous growth. Shoots on these trees had also been trimmed back to 3 or 4 buds each time they reached 15-20 cm in length.

Shoots approximately 25 cm long were collected and leaves were removed leaving only 1 to 2 cm of the base of the petiole attached to the stem. Shoots were briefly rinsed in running tap water, then cut under water into nodal segments 3 to 5 cm long. Segments were washed for 3 hours under running tap water and then surface-sterilized in 1% sodium hypochlorite (20% household bleach) plus 1 to 2 drops of Tween 20 per liter for 10 to 20 minutes, depending on the tenderness of the pieces. Segments were then individually rinsed with sterile water 3 times and placed in shell vials on the corrected Driver-Kuniyuki-Walnut (DKW) medium. (The medium as originally published contained several errors). Unless otherwise noted, culture vessels were maintained at room temperature under 24 hr cool white and Gro and Sho (General Electric) plant growth fluorescent lights (55-110 \(\mu E*\text{m}^{-2})*\text{s}^{-1}\).

Explants were maintained on basal DKW for the first 2 days and then were transferred to DKW with hormones (BA 1.0 mg*liter\(^{-1}\)) and indole butyric acid (IBA) 0.01 mg*liter\(^{-1}\). A 2 to 3 day transfer schedule was maintained for the first 3 months in culture. Three to 6 months after initiation, transfer frequency was gradually reduced to 1 week intervals. Explant bases were given a fresh transverse cut at least once a week beginning at week 2. All new leaves were kept on the explants and between 2 and 6 weeks explants were transferred from shell vials to GA-7 vessels (Magenta Corp, Chicago, IL) to provide room for full leaf development.

Eight to 10 weeks after initiation, apical portions (3 to 4 cm) of the new shoots which had emerged at least 4 cm from the primary bud of the original explant and which had swollen axillary buds were excised and maintained as independent cultures. This excision stimulated shoot formation from the secondary bud of the explant and enlargement of the axillary buds on the basal portion of the primary shoot, which then developed axillary shoots.

Beginning 6 months after initiation, shoots were excised from cultures, placed on a pre-rooting medium and were maintained for 1 week at 18°C under 16 hr cool white fluorescent light [35-60 \(\mu E*\text{m}^{-2})*\text{s}^{-1}\)]. Shoots were then washed, dipped in 2% IBA in talc powder (Fisher), and placed in peat plugs (Castle and Cooke, Techniculture, Inc., Salinas, CA 93902) under the same light and temperature regime. After 1 week in plugs, shoots were washed again, potted in Supersoil (McLellan Co., So. San Francisco, CA 94088) in enclosed containers, and placed under 16 hr cool white light (75-150 \(\mu E*\text{m}^{-2})*\text{s}^{-1}\) at room temperature. Acclimatization was achieved by gradually opening the containers over a 4 week period. Rooted shoots were placed in the field for evaluation of cultivar behavior on their own roots. A direct field-rooting procedure was also successfully used to obtain micropropagated plants in the field.
RESULTS AND DISCUSSION

In this study, 36 'Sunland' (Su) and 24 'Vina' (Vi) explants were introduced into culture. Of these, 48% (18/36 Su, 11/24 Vi) remained free of contamination after 4 weeks in culture. Axillary shoots were produced on 56% (13/18 Su, 7/11 Vi) of the sterile explants and 11 (6 Su, 5 Vi) of these were successfully subcultured to produce repetitively multiplying cultures. Rooting occurred with 5 to 75% success after 4 to 12 weeks. Currently 5 'Sunland', 5 'Vina' and over 100 'Chandler' plants from in vitro propagation are in soil.

Successful culturing of mature material was achieved by modifying explant handling and culturing techniques rather than by altering media composition. The use of a running water wash and individual rinses after sterilization increased 4 week survival of explants to 48% from the 13% achieved in preliminary studies which lacked these modifications. Maintenance of explant size and retention of leaves were important in promoting explant survival and growth. During the initial 2 months in culture, explants with leaves partially or entirely removed were less vigorous and showed a greater tendency to callus than explants with leaves intact.

Slow decline, characterized by lack of growth, chlorosis, and leaf abscission was overcome by rapid transfer and basal trimming of explants. During the initial 3 months in culture, extension of the transfer interval to 3 days resulted in explant chlorosis. Similarly intervals exceeding 7 days between shoot base trimmings were detrimental.

During the initial 3 to 4 months in culture, growth was slow and leaf structure was relatively large and coarse. After this period a change in the explant morphology became apparent. This was best observed as a decrease in leaf size and increased tractability in culture. The smaller, finer featured leaves signalled a reduced sensitivity to explant size requirements, increased growth rate, increased multiple shoot production, and an ability to sustain longer transfer intervals. Within a month of this change, the cultures could be maintained on 1 week transfer intervals and were readily multiplying by both multiple shoot production and shoot division. This shift is not considered a reversal to a juvenile phase because several of these cultures have flowered after 10 to 15 months in culture.

The 3 cultivars described in this paper are now being multiplied in a commercial laboratory. This method is currently being applied to 'Hartley' walnut and appears successful.