EVALUATION OF PEPPERMINT FIELD PERFORMANCE FROM PLANTS REGENERATED FROM MERISTEM TIP CULTURE

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Abstract

Observations in Montana revealed that rooted cuttings produced from privately-produced meristemmed peppermint (cv 'Black Mitchum') grew more vigorously than the nonmeristemmed certified rooted cuttings from which they were taken. We (1) obtained greenhouse rooted cuttings from both sources for field evaluation in central Oregon, (2) conducted a literature review on the subject with respect to mint, and (3) initiated a meristem program at the Central Oregon Agricultural Research Center (COARC). The scientific literature indicated that peppermint has been successfully meristemmed in many laboratories, but there was no report of enhanced growth as a result of meristemming. Results of first-year growth of cuttings planted in test plots are presented: Rooted meristemmed cuttings arrived much larger and robust than the nonmeristemmed rooted cuttings, which could have been a result of differences in age and management of the sources of the cuttings as well as any effect of meristemming itself. In replicated field trials, differences in growth persisted for several weeks in the field, but over the course of the summer of 1992 growth differences diminished. By August 1992, no differences were noted between the two plantings. This suggested that earlier growth differences were most likely due to greenhouse handling rather than due to effects of meristemming, nor were they due to the small chance that a genetic change occurred during the meristemming and plant regeneration phase. These results do not explain the observed differences between meristemmed and nonmeristemmed peppermint plants in Montana, and do not eliminate some possible complex pathogenisis that could be different between Montana and central Oregon. The initial steps in the meristemming and plant regeneration process, which seemed relatively routine, were conducted at the COARC.

Introduction

Meristem tip culture has been widely used since the early 1960's, as a tool to eliminate viruses (and occasionally bacteria and other systemic pathogens) from vegetatively-propagated plants. In general, systemic pathogens lag behind the growing points of plants, primarily because they move most quickly in developed vascular tissues that are not developed in the very tip region of the growing points (the meristem area). Mint is not known to harbor viruses routinely in planting-stock, either greenhouse rooted cuttings or field stock however, viruses do exist in many vegetatively propagated species that may not manifest known symptoms. When these viruses are removed, the virus-free materials may

grow dramatically different, or the response may be more subtle. Sometimes no obvious growth differences occur, but the virus-free material may be more hardy or able to resist stresses or other infections. It is rare that no effect is seen following removal of a virus from vegetatively-propagated stock. Sometimes, meristem tip culture has shown the prior presence of a virus that was previously unknown to be present. In general, reports of improvement in performance of a vegetatively-propagated plant following meristem tip culture suggest that a virus or other systemic pathogen may have been present previously. Other techniques can be employed to confirm this.

Mint has been meristemmed successfully in various laboratories (1,3,4) with no report of enhanced virus. A number of observers have witnessed remarkable growth of peppermint in Montana from plants that were regenerated from meristem tips of 'Black Mitchum' variety. To date, all such meristemmed plants have come from one commercial greenhouse, and we are aware of no direct comparison plantings of this material with nonmeristem plants. In response to a request by the Oregon Mint Commission, we obtained plants from the known commercial source of meristemed plants, and another commercial source of non-meristemed plants of the same variety. There potentially could have been a problem with receiving material with substantially distinct management histories in the rooted cutting and greenhouse production process (light, potting medium, fertility, etc.), which could influence plant behavior for some time after planting in the field. It was decided to proceed with what material could be obtained in 1992, irrespective of these problems.

In addition, we planned to develop our own meristemmed plants in our laboratory, so that these could be managed identically to the nonmeristemmed plants. Also, we could obtain information on the relative ease of meristemming various varieties.

Materials and Methods

A thousand rooted cuttings, each of meristemmed and nonmeristemmed peppermint (Black Mitchum' variety), were obtained from two different commercial sources for comparison of growth in the field in central Oregon. Ideally, nonmeristemmed plants would have been the mother source for meristemmed plants, and rooted cuttings from each would have been produced in the same greenhouse system to reduce variation in field performance due to genetic or management practices. However, this was not possible in 1992.

Plants were shipped and received at the same time and planted within two days into replicated field plots at the COARC, Madras field in early summer, 1992. Plants from each source were substantially different in size and development at time of arrival and planting. Meristemmed plants received from Starkle Farms Inc., Montana, were quite large (13-15 inch single stems, root system length 5-6 inches, 7-9 leaf nodes, 1-5 rhizomes plus stolons), and presumably had been allowed to develop in the commercial greenhouse prior to shipment. Nonmeristemmed plants received from Plant Technologies, Inc., Oregon, were from relatively recent cuttings and were smaller (3-4 inch single stems, 3-4 inch root system length, 6-7 leaf nodes, 0-1 rhizomes plus stolons). To sooner judge post-planting growth

free of greenhouse management influences, the top growth of meristemmed plants was cut back to 4-5 inches in length just prior to planting. Two hundred and fifty plants were spaced one foot apart in rows 2 feet apart, in 10 rows per plot, on June 1, 1992. The field location had no prior mint production and was prefertilized. The systemic insecticide aldicarb was applied to all plots to inhibit spread of viruses in plots. Plantings were hand weeded for six weeks, and then treated with 0.75 lbs/a of Sinbar. Plantings were irrigated 2-3 times/wk for one month and 1-2 times/wk thereafter through October 1992 via overhead sprinklers.

Results and Discussion

All rooted cuttings suffered from heat stress and desiccation during the first several days and weeks of establishment during the very hot summer of 1992. There were more failures among meristemmed than among nonmeristemmed plants (3 vs. 1 percent). Growth among meristemmed plants was more vigorous early in establishment than among nonmeristemmed plants, but as plants developed between late June and late August, growth in all plots converged to the point that meristemmed plants could not be distinguished from nonmeristemmed plants. Continued growth and development (plant height, rhizome and stolon development, and overall appearance and vigor) seemed similar since late August. In all ways, mint growth in all plots seemed what we expected of rooted cuttings, and was similar in performance to `Black Mitchum' elsewhere on the COARC Madras farm. Plots were not harvested.

No verticillium wilt symptoms were observed in any plots. A few plants within both meristemmed and nonmeristemmed plots (six plants total) declined rapidly as temperatures cooled during September and October, but did not manifest typical verticillium wilt symptoms. Each of these six plants tested positive for Tomato Spotted Wilt Virus-Impatiens Strain [TSWV-I], but symptomless plants from the same plots tested negative. The source of TSWV-I infection is not known, but the presence of this virus in this trial does **not** account for the general growth convergence and similarity among meristemmed and nonmeristemmed plants.

Plots will be maintained into 1993 for further observations, but based on growth in 1992 there seems to be little support for the hypothesis that the particular meristemmed plants received in 1992 might grow substantially better than nonmeristemmed plants perhaps this is due to some undetermined systemic pathogen (virus or bacteria). Reports of unexplained and exceedingly vigorous growth in Montana for meristemmed plants are not challenged by current results in this trial, but suggest that such growth may be due to other factors than a system pathogen that is eliminated by meristem tip culture. It would have been worthwhile to conduct this same trial in Montana at the same time to determine if there were growth differences there during 1992 between these two sources of rooted cuttings.

There is always the possibility that other sources of rooted cuttings may have performed differently than the ones evaluated in 1992. Various sources of `Black', Todds', and redefined and old `Murray' cuttings were collected from both central Oregon and the

Willamette Valley in the fall of 1992, both normally appearing sources and some which were thought to grow differently. These were to be meristemmed in the fall-winter of 1992-93, and rooted cuttings from nonmeristemmed and meristemmed sources of each were to be grown in the field in 1993 to determine whether growth differences may occur that might be attributed to an undetermined systemic pathogen. At this time, these plants will be held in isolation in small field plots until after spring growth is evaluated in the meristem/nonmeristemmed plots. If there seems little reason to proceed, these plants will be destroyed. One off-shoot of this program may be a determination of how easily various varieties may be meristem tip cultured. Preliminary results in our laboratory suggest that peppermint meristems respond quite well to standard methods of regeneration of plants (Murashige, I., and F. Skoog. 1962), as was suggested by the reported success of various laboratories around the world (Holm, Y., R. Holtunen, K. Jokinen, and T. Tormala., 1989, Repcakova, K., M. Rychlova, E. Cellarova, and R. Honcariv., 1986, Rodov, V.S., and O.A. Davidova., 1987).

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