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

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Abstract

'Black Mitchum' peppermint, commercially meristem tip cultured (M) and then propagated by rooted stem cutting was compared to the same variety (of the original mother stock to M plants) propagated by rooted stem cutting (Non-M). Rooted cuttings of each type were placed into a randomized, complete block experimental design field trial in 1992. In 1993, M plants initially were more vigorous through the first half of the season. Main stems were 15 percent taller and more consistently upright throughout the season. Total above-ground plant dry weight was 17-40 percent greater in M plants compared to Non-M plants. The superior total dry weight advantage for M plants was stem weight. Non-M plants had a greater number of branches and total leaves, greater total leaf weight, and less stem weight than M plants. Though stem thicknesses were similar for both M and Non-M plants, the leaf-to-stem ratio was much greater for Non-M plants than for M plants. This likely explains the earlier and more extensive lodging of Non-M plants through July, in contrast to M plants which remained upright until just prior to harvest in mid-August. For three harvest dates in August, oil yield from plots with Non-M plants was 16-55 percent greater compared to plots with M plants. Oil composition was similar between treatments, except for total heads, which was higher for Non-M than for M plants. These results are discussed with respect to management practices that might improve performance of M plants while taking advantage of the vigor shown following meristem tip culturing.

Introduction

Meristem tip culture of plant materials was introduced in the 1960s. It is a process where a growing point of a plant is extracted prior to development of the conductive tissues, and a new plant is then regenerated (3). With this process, genetic identity is retained but vascular pathogens, such as viruses and certain bacteria and fungi that move quickly through vascular tissues, can be eliminated (7). [Vascular tissues are those which allow long-distance transport of water and nutrients around the plant.] Meristem tip culture has proven most beneficial for development of pathogen-free planting materials for vegetatively-propagated crops (bulbs, cloves, tubers, corms, crowns, rootstock, etc.) where vascular pathogens commonly are retained in the planting material from generation to generation. In most cases, 'when vascular pathogens are so eliminated, plant materials respond by growing without disease symptoms and/or with greater vigor. If increased vigor occurs following meristem tip culture, a vascular pathogen present in the stock that the meristem tip cultured plants were derived, even when clear disease symptoms were not apparent.

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This research project was developed in response to observations that 'Black Mitchum' peppermint, commercially meristem tip cultured in Montana, was growing more vigorously when field planted in Montana compared to other Montata plantings. A review of the scientific literature indicated peppermint and other mint species had been repeatedly meristem tip cultured and regenerated in various laboratories around the world, following standard practices for inducing roots and shoots in culture (1,4,5). None of these reports indicated a growth enhancement following this procedure, although not all reports included field planting of the regenerated material. None of these reports were from laboratories from agricultural centers in North America, nor were standard commercial North American varieties used.

Materials and Methods

Rooted cuttings of 'Black Mitchum' peppermint derived from commercially meristem-tip cultured (M plants, specific procedures undefined) were received from Starkle Farms, Inc., Ronan, MT. Similarly, rooted cuttings from non-meristem tip cultured plants (Non-M plants) were obtained from Plant Technology, Inc, Albany OR. Both materials arrived as boxed cuttings on June 30, 1992. Stem length of rooted cuttings of M plants were 8-12 inches above roots, with 1-3 side branches. Stem length of Non-M plants was 4-6 inches above roots, with no side branching. Root masses of M rooted cuttings were 2-3 times the volume of Non-M plants. Leaves on M plants were larger than those on Non-M plants.

In discussion with the two propagators, it was clear that greenhouse handling of each of the two was somewhat different, and that M plants had been held longer since cuttings were taken than had Non-M plants. On this basis of differential handling, it was anticipated that initial growth in the field following planting would likely be different for the two sources, and that the initial effect of meristem tip culturing could not be distinguished from effects of handling practices.

Planting was on July 1, 1993. Just prior to planting, rooted cutting of M plants were trimmed to 6 inches, and roots were trimmed to match root length of Non-M plants. Non-M rooted cuttings were planted without trimming. Opened furrows were 4-inches deep. Plants were placed in the furrows and immediately covered, with 2 inches left above the soil line. In each of 25-ft x 25-ft square plots, 250 rooted cuttings were planted one per foot into ten 25-ft opened furrows spaced 2 ft apart. Four plots of each treatment (M and Non-M) were arranged in a randomized, complete block experimental design. Plots were separated by 6-ft unplanted alleys. Plots were immediately irrigated with solid set irrigation, and regularly irrigated and fertilized equally as per commercial practices through the course of the study. Plots were hand-weeded through the early fall of 1993, when a half-rate of "Sinbar" herbicide was applied. Beginning in 1993, all weed control, fertility and irrigation was as per standard commercial practice for the peppermint in central Oregon. No insect or mite control was required in either 1992 or 1993.

Plots were harvested with a 40-inch wide plot forage harvester, and sub samples were collected for oil distillation in research stills located at the Central Oregon Agricultural Research Center. Oil was distilled within 10 days of harvest from hay which had been airdried from about 10 pounds of fresh hay. Distillation was in small research stills, with exit temperature of condensate held between 110-120°F and as close to commercial procedures as could be followed (2). Oven-dry weight of harvested hay was calculated based on the total fresh harvested weight. Other data were collected as indicated below. Data were analyzed by analysis of variance.

Results

Results from 1992: Mid-day temperatures immediately following planting were near 100° F during the two weeks following planting. All stems and foliage left above ground desiccated. New top growth appeared later in July, and stands were evaluated in early August. Stands were about 95 percent for all plots. Growth continued into October, 1992. Initial growth was slightly more vigorous on M plants in July and early August, but growth was determined to be equal after about mid-August. Rhizomes developed in late August and September in all plots. Plots were not harvested in 1992.

Results from 1993: Early spring prostrate growth was similar for mint in all plots. Beginning in mid-May, growth began to appear taller and more vigorous in plots with M plants. Beginning in early June, various types of data were collected to compare mint growth and performance in plots. For plant height, branching, leaf dry weight, stem dry weight, and stem thickness, forty plants were selected at random throughout plots, excluding a one-foot perimeter where plant growth was unrepresentative of growth internal to the plot. Estimates of lodging were taken by visual rating of the entire plot, again excluding a perimeter one foot. Harvest was as described above. Fresh weight of hay collected from 40-inch x 25-ft strips was taken. Sub-samples were collected for dry weight and oil measurements.

Results are expressed in a series of Tables 1-6. M plants were consistently more vigorous. This is reflected by a 15 percent height advantage (Table 1), greater total dry weight through the growing season (Table 4) and 17-40 percent greater total dry weight at harvest (Table 5). However, this vigor was not channeled into plant components which resulted in improved commercial importance. Non-M plants, although shorter (Table 1), branched more extensively and developed more leaves (Tables 2 and 4). Non-M plants were of equal stem thickness (Table 3), but because more of the weight of Non-M plants was in leaves rather than stem (Table 4), Non-M plants lodged earlier and more extensively than M plants (Table 3). At harvest M plants had predominantly older leaves, many of which fell off due to delayed harvest.

Because there was much more of the plant as leaves in Non-M plants than found for M plants, oil yields favored Non-M plants (Table 5).

Oil composition was determined by Wm. Leman, Inc. Components evaluated included total heads, total menthol, menthofuran, and esters. For both M and Non-M treatments, the relative composition of each component changed with each harvest date. This difference with respect to harvest date was statistically significant at the 1 percent level for each component. Considering all plots for harvest dates of August 9, 12 and 16, total heads averaged 10.5, 8.3 and 9.3 percent; total menthol averaged 50.4, 55.2 and 52.7

percent; menthofuran averaged 3.2, 4.0 and 6.9 percent; and esters averaged 6.6, 8.0, and 7.7 percent respectively. The only component which was statistically different between M and Non-M treatments was total heads (Table 6), with the M treatment consistently less than the Non-M treatment for each harvest date.

With each delay in harvest date, M plants visually appeared to suffer greater drought stress than Non-plants. At the third harvest date, all plants in all plots were drought stressed, but M plants more so, which likely relates to the enhanced leaf drop in plots with M plants.

Discussion

The relatively poor performance (20-50 percent less oil yield combined with 20-40 percent greater hay weights to handle) of meristem tip cultured plants in this trial in 1993 apparently also was reported to have been experienced in commercial plantings in Montana, Washington, and other states, where growers have planted this material in anticipation of improved performance. Two caveats must be kept in mind: first, one year's performance may not reflect long-term performance. Second and more importantly, the growth differences might be manageable. For example, rolling the mint in July to induce branching and secondary growth might result in greatly improved performance. Or perhaps clipping the primary stems might similarly induce side branching. Perhaps the increased vigor could lead to a double cutting system which may not result in poor vigor the following year, which frequently results when non-meristem tip-cultured mint is double cut.

Enhanced growth of plants which had been meristem tissue-cultured suggests either that (a) some unknown vascular pathogen may have been eliminated, or (b) that a genetic change may have occurred in the meristem process. Several cursory efforts to detect a viral particle in Non-M 'Black Mitchum' plants using an electron microscope so far have been negative (T. Allen, Oregon State University, and William Grey, Montana State University, personal communication). However, virologist (D. Mayhew, California State Dept. Agr., Sacramento, personal communication) has indicated visualization using transmission electron microscopy of a long ,flexuous rod, closteroirus-like particles in a sample of Oregon-grown mint. When thin-sectioned, a large accumulation of virus-like particles were observed in the vascular tissue. Preliminary efforts to transmit a virus mechanically or by grafting were not successful. No further efforts are in progress at this time.

The likelihood of a genetic change seems small, but remains possible. Apparently, natural selections of Japanese mint *(Mentha arvensis)* may vary in ploidy, which affects growth, yield and oil composition (6). If meristem tip-culturing is done in a normal manner, usually such chromosomal changes will not occur. The extent that wild or cultivated peppermints may vary in this respect is undetermined.

Conclusions

It is recommended that verification studies be conducted to determine if a vascular pathogen is present in most or some field-grown peppermint. Verification of such a pathogen or pathogens, and the types, would help direct future meristem tip-culture efforts, verification would also shape any organized program to develop pathogen-free materials on an industry-wide basis, and would assist efforts to investigate field epidemiology of any such pathogens. Should systemic pathogens not be found, it may be prudent to conduct a cytogenetic analysis of meristemmed tissue to determine if major chromosomal translocations or even ploidy changes may have occurred. These analyses could proceed sequentially or together.

It is not clear that mint, which is freed of a vascular pathogen, will remain pathogen free. Many other such pathogen-free plant materials become re-infected via insect or other vectors, or mechanically during routine handling (7).

Our plots will be maintained for several years to evaluate long-term comparative performance, of meristemmed and non-meristemmed mint, and to determine if the plots retain the vigor over time (should some vascular pathogen become reintroduced into the planting). The plots are suitably sized, such that a single management practice (e.g. rolling) could be introduced for further comparisons.

References

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TABLES

Table 1. Height (cm) of main stems in 'Black Mitchum' peppermint either meristem tip cultured (M) or not meristem tip-cultured (Non-M), located in a replicated field trial at the Central Oregon Agricultural Research Center, Madras OR, 1993. [Means of 40 plants selected at random from each of 4 randomized and replicated plots].

Treatment	June 8, 1993	July 12, 1993
Meristem	56.9	84.5
Non-meristem	49.2	73.4
Significance Level	4%	1%

Table 2. Number of branches and total leaves of 'Black Mitchum' peppermint either meristem tip cultured (M) or not meristem tip-cultured (Non-M), located in a replicated field trial at the Central Oregon Agricultural Research Center, Madras OR, 1993. [Means of 40 plants selected at random from each of 4 randomized and replicated plots].

Treatments	Number of Branches per Main Stem on July 12, 1993	Total Number of Leaves on Main Stem and Attached Branches on July 12, 1993
Meristem	26.1	21.2
Non-meristem	32.8	26.1
Significance Level	10%	5%

Table 3. Stem thickness (cm) and percentage stems which had lodged of 'Black Mitchum' peppermint either meristem tip cultured (M) or not meristem tip-cultured (Non-M), located in a replicated field trial at the Central Oregon Agricultural Research Center, Madras OR, 1993. [Thickness values are means of 40 plants selected at random from each of 4 randomized and replicated plots, lodging is an estimated visual rating for entire plots].

Treatment	Main Stem Thickness on July 23, 1993	Percentage Lodging on July 26, 1993
Meristem	1.27	50
Non-meristem	1.24	98
Significance Level	Not Sig	<1%

Table 4. Dry weight (gm) of foliage and stems of 'Black Mitchum' peppermint either meristem tip cultured (M) or not meristem tip-cultured (Non-M), located in a replicated field trial at the Central Oregon Agricultural Research Center, Madras OR, 1993. [Total oven dry weight of 40 plants selected at random from each of 4 randomized and replicated plots]

Treatment	Dry Wt Leaves (L), July 28, 1993	Dry Wt Stems (S),July 28, 1993	Dry Wt L+S, July 28, 1993	L/S Dry Wt Ratio, July 28, 1993
Meristem	62.7	86.9	149.5	0.726
Non-meristem	68.2	73.5	141.7	0.924
Significance Level	Not Sig	Not Sig	Not Sig	<1%

Table 5. Oil yield and dry weight yields of 'Black Mitchum' peppermint either meristem tip cultured (M) or not meristem tip-cultured (Non-M), located in a replicated field trial at the Central Oregon Agricultural Research Center, Madras OR, 1993. [Derived from 25 ft x 40 in harvested strip, and distillation from approx. 10-lb subsample of fresh hay.]

OIL YIELD, LBS/AC	Harvest 1, Aug 9, 1993	Harvest 2, Aug12, 1993	Harvest 3, Aug 16, 1993	Average All Harvests
Meristem	57.7	57.8	39.9	51.8
Non-meristem	71.6	67.2	61.8	66.8
Significance Level	1%	1%	**1%**	1%

DRY WEIGHT YIELD, LBS/AC	Harvest **1**	Harvest 2	Harvest 3	Average All Harvests
Meristem	9812	7815	5899	7841
Non-meristem	7579	6671	4199	6149
Significance Level	Not Sig	Not Sig	Not Sig	Not Sig

[Note: There was additionally a statistically significant harvest date effect ($P<0.01$): dry weight dropped with later harvest date, due to increased loss of leaves

OIL/DRY WT RATIO	Harvest 1	Harvest 2	Harvest 3	Average All Harvests
Meristem	0.60	0.77	0.67	0.68
Non-meristem	0.96	1.06	1.54	1.19
Significance Level	5%	5%	5%	5%

[Note: There was a statistically significant harvest date x treatment interaction ($P<0.05$): The oil to dry weight ratio increased more in non-meristemmed mint than meristemmed mint. Meristemmed mint was observed to lose leaves disproportionately to meristemmed mint.]

Table 6. Oil compositional character "total heads" (%) from 'Black Mitchum' peppermint either meristem tip cultured (M) or not meristem tip-cultured (Non-M), located in a replicated field trial at the Central Oregon Agricultural Research Center, Madras OR, 1993. [Oil analyzed by Wm. Leman, Inc.]

TOTAL HEADS	Harvest 1, Aug 9, 1993	Harvest 2, Aug 12, 1993	Harvest 3, Aug 16, 1993	Average All Harvests
Meristem	10.3	8.1	8.8	9.1
Non-meristem	10.7	8.6	9.4	9.6
Significance Level	5%	5%	5%	5%

[Note: Differences among harvest dates were statistically significant at the 1% level.]