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Comparative salt responses at cell and whole-plant levels of cultivated and wild tomato species and their hybrid

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SUMMARY

Response to increased salinity was compared in whole plants and calli from leaf, stem and root of *Lycopersicon esculentum* Mill. cv. P-73, *Lycopersicon pennellii* (Correll) D'Arcy ac PE-47 and their interspecific hybrid. Three NaCl treatments were applied (0, 70 and 140 mM) for 28 d. In both calli and whole plants, *L. pennellii* was more salt-tolerant than the cultivated species and the hybrid according to the growth responses, although different degrees of salt tolerance were generally found between plants and calli. The Na^+ and Cl^- accumulations with salinity were higher in *L. pennellii* than in *L. esculentum* at both levels of organization. The interspecific hybrid showed an accumulation ability for Na^+ and Cl^- intermediate to its parents in the shoot of the whole plants and similar to *L. pennellii* in the callus tissues. Either no decrease or a small decrease of K^+ concentrations with salinity were found in both whole plants and callus tissues of *L. esculentum*. However, K^+ concentrations decreased in the organs and calli of *L. pennellii* and the hybrid with increasing salinity. Only at the whole plant level did *L. pennellii* have a Na^+/K^+ ratio higher than *L. esculentum*, showing the hybrid to have values between those of its parents in the shoot.

As salinity is a major factor limiting crop productivity in the semi-arid areas of the world, wild genotypes with a high degree of salt tolerance might be useful in breeding programmes to improve plant yield under salinity. The tomato is a good dicotyledoneous plant model for salt tolerance studies (Tal, 1984), but the introduction of wild traits into cultivated species and the selection of hybrids are hampered by a lack of efficient screening methods so that cell culture techniques might allow a more rapid evaluation of the salt-resistance of the crosses, providing that a relation is observed between salt tolerance at both cell and whole-plant levels (Dracup, 1991).

In some glycophytes, including the tomato, a positive correlation between growth of calli and whole plants has been observed in saline conditions (Tal *et al.*, 1978; Smith and McComb, 1981), suggesting that either whole plants or callus tissues could be used to investigate salt responses. However, further studies on tomato (Bourgeais *et al.*, 1987; García-Reína *et al.*, 1988a) have indicated that salt-tolerance of callus was dependent on the explant source from which the calli were derived.

To evaluate the validity of the selections at the cell level in a tomato breeding programme for salt-tolerance, and to identify the main physiological traits of the wild or cultivated parents in the cell/whole plant responses of tomato hybrids, we report on the growth responses and some aspects of mineral nutrition of calli and whole plants of cultivated and wild tomato species and their hybrid.

MATERIALS AND METHODS

Whole plant culture

Seeds of *Lycopersicon esculentum* Mill. cv. P-73, *L. pennellii* (Correll) D'Arcy accession PE-47 and their hybrid *L. esculentum* × *L. pennellii* were sown and the resulting plants grown in washed silica sand under controlled conditions in a controlled culture chamber, as described previously (Pérez-Alfocea *et al.*, 1993). The plants were irrigated with Hoag-

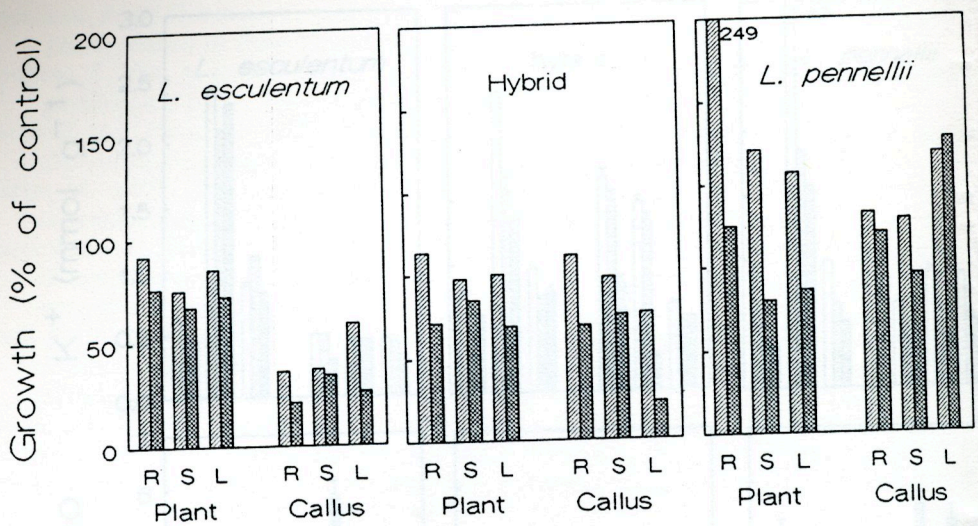

FIG. 1
Relative growth of the plant organs (R, root; S, stem, and L, leaf) and the calli proceeding from root, stem and leaf of *L. esculentum*, *L. pennellii* and their hybrid exposed to 70 (▨) and 140 mM NaCl (▩).

land's solution. Salt treatments were applied to 25 d old plants using 0, 70 and 140 mM NaCl nutrient solutions for 28 d. Then the plants were cut into root, stem and leaf fractions and the fresh and dry weights determined. Three replicates (eight plants per replicate) were used per treatment.

Callus initiation and culture

Calli were initiated from 5 mm segments of roots, stems and leaves from 12 d old seedlings of each population, on a culture medium including MS mineral nutrients (Murashige and Skoog, 1962) and (in mg l^{-1}): inositol, 100; thiamine, 1; nicotinic acid, 1; pyridoxine, 1; glycine, 2; naphthalene acetic acid, 0.5; kinetin, 0.5; agar, 8,000; and sucrose, 30,000. Calli were subcultured every four weeks on medium prepared as that of initiation, except for the growth regulators (2,4 dichlorophenoxyacetic acid, 0.5 and kinetin, 0.5). In the fourth subculture, the NaCl treatments (0, 70 and 140 mM) were applied for 28 d. Three replicates of 10–15 calli were used. The relative growth rate (RGR) of calli was measured by the $(m_f-m_i)/m_i$ ratio, where m_i and m_f were the fresh weights of calli at the time of the transfer and after 28 d of culture, respectively.

Analyses

The ion contents were determined in roots, stems and leaves of the whole plants and their corresponding calli according to Pérez-Alfocea *et al.* (1993). Briefly, Na^+ and Ka^+ were determined by atomic absorption spectrophotometry after digestion of dry matter with a mixture of nitric-perchloric (2:1 v/v) acids. Cl^- was determined by potentiometric titration with $AgNO_3$ from the aqueous extract. Ion contents are expressed on the basis of dry weight.

RESULTS AND DISCUSSION

Growth

The organs and calli of *L. esculentum* were generally more salt-sensitive than organs and calli from *L. pennellii* (Figure 1), as previously found by Tal and Shannon (1983) at the whole plant level. Only at 140 mM NaCl, were there no significant differences between shoot growth reductions of both tomato species. In the hybrid, the plant growth reductions with salinity were more similar to that of *L. esculentum*; in the callus tissues, however, the salt tolerance of the hybrid was intermediate between those of the parents, except in the calli from leaf, which showed a response similar to that of *L. esculentum*.

Generally, the callus RGR and plant biomass were not strictly the same. The stem and root dry weights and the RGR of their corresponding calli were affected similarly under saline conditions only in the hybrid (Figure 1). The

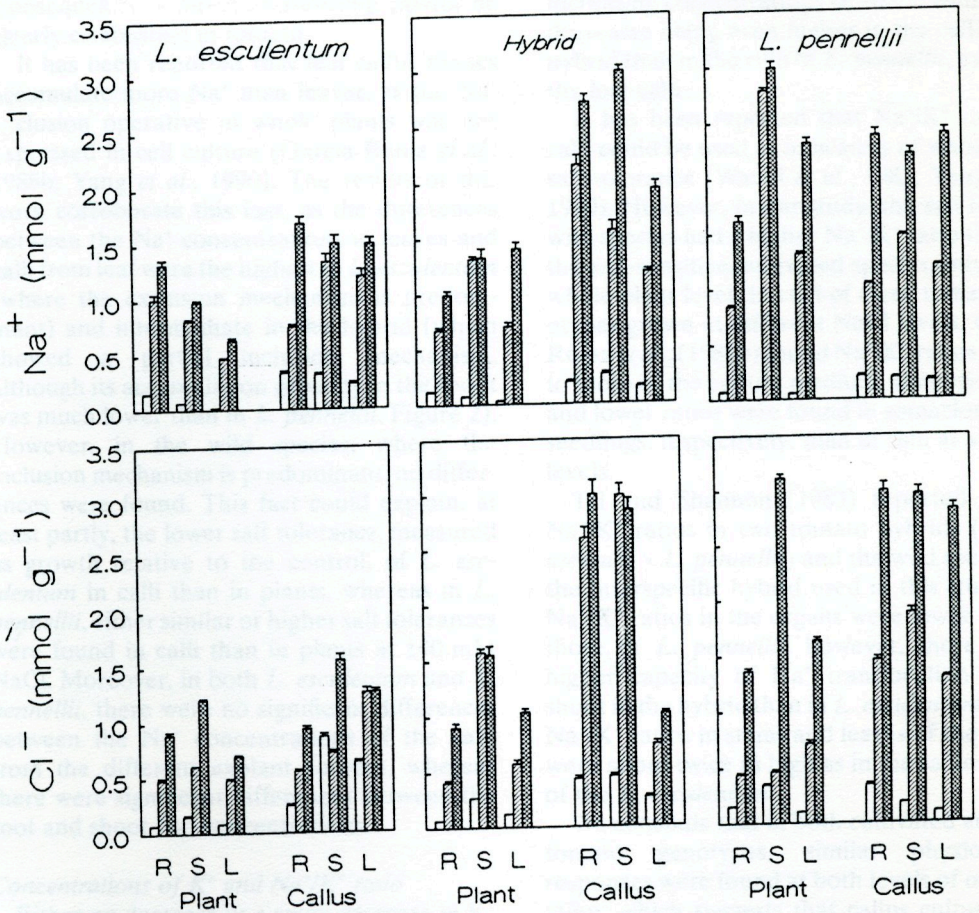

FIG. 2
Na^+ and Cl^- concentrations in both whole plants (R, root; S, stem, and L, leaf) and calli (from root, stem, and leaf) of *L. esculentum*, *L. pennellii* and their hybrid grown in absence of NaCl (□) and exposed to 70 (▨) and 140 mM NaCl (▩). Bar indicates ± SE.

calli of *L. esculentum* obtained from the three explant sources and the calli from leaves of the hybrid were more salt-sensitive than their corresponding organs. In contrast, calli from leaves of *L. pennellii* were more salt-tolerant than the leaves of the whole plants. Because the same culture media and nutrient solutions were used, respectively, for all the calli and all the plants, the salt responses did not result from the low osmotic potential of the basal media nor the influence of the culture medium components (Dracup, 1991). Changes in growth responses between calli could be linked, at least partially, to explant source, as was the case in the hybrid, where the calli from leaves appeared more salt-sensitive than the calli from stems and roots. Van den Bulk *et al.* (1990), using different explant sources (cotyledon, leaf, hypocotyl) in tomato, reported the possibility of recovering mutations affecting the frequency of polyploidy and therefore the chromosome variations. This somaclonal variation could also contribute to a modification of the degree of salt-tolerance.

Concentrations of Na^+ and Cl^-

The shoot of *L. pennellii* accumulated high amounts of Na^+ and Cl^-, while *L. esculentum* excluded them from the leaves (Figure 2). The Na^+ and Cl^- concentrations in the shoot of the hybrid were higher than in *L. esculentum*. This suggests that the 'excluder' behaviour shown by *L. esculentum*, such as exclusion and/or retention in the root and restriction of sodium trans-

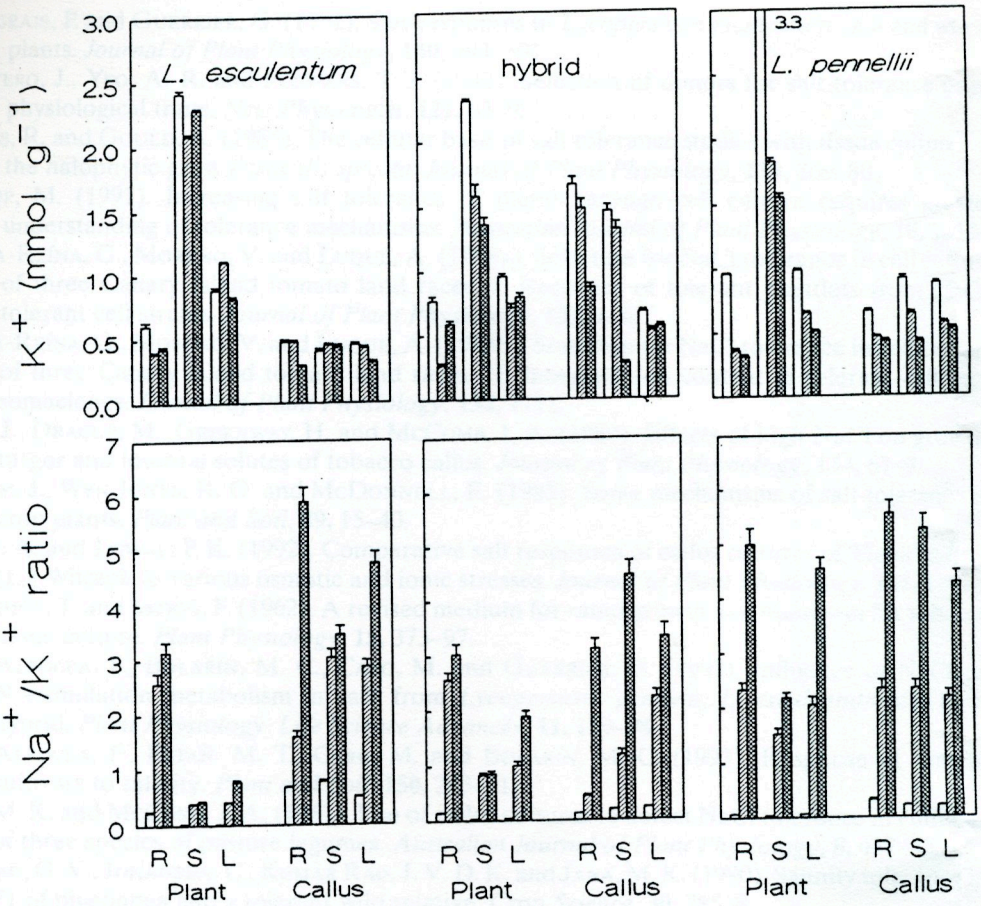

FIG. 3
K^+ concentration and Na^+/K^+ ratio in both whole plants (R, root; S, stem, and L, leaf) and calli (from root, stem, and leaf) of *L. esculentum*, *L. pennellii* and their hybrid grown in absence of NaCl (□) and exposed to 70 (▨) and 140 mM NaCl (▩). Bar indicates ±SE.

location to the shoot (Pérez-Alfocea *et al.*, 1993) partially disappears in the hybrid. Tal and Shannon (1983) and Subbarao *et al.* (1990) reported wild-type physiological traits in the hybrids, suggesting that these traits were inherited and controlled by a dominant gene(s).

Comparisons between the nutrient contents of calli were possible, because they were morphologically similar in the control conditions (Perez-Alfocea *et al.*, 1992) and, consequently, the solute contents of their free spaces were similar (Bourgeais and Guerrier, 1992). The differences in structure of calli under control and saline conditions (salt media increased the compaction of calli) resulted in differences of solute accumulations between and within cell walls (Gibbs *et al.*, 1989).

The leaf, stem and root calli of *L. esculentum* accumulated less Na^+ and Cl^- than the tolerant tissues of the wild species, especially at 140 mM NaCl, which suggests a direct relationship between salt tolerance, measured as growth, and the concentrations of Na^+ and Cl^-. These results obtained with tomato differ from those in other plant species, as there was either an inverse relationship between growth and inorganic solute concentrations (*Vigna radiata*, Gulati and Jaiwal, 1992) or similar Na^+ contents in adapted and non-adapted cells of *Nicotiana* and *Distichlis* (Watad *et al.*, 1983; Daines and Gould, 1985). However, the root and stem calli of the hybrid, which were less salt-tolerant than that of *L. pennellii*, showed higher accumulations of Na^+ and Cl^- than those of *L. pennellii*.

Consequently, a direct relationship cannot be clearly established in tomato.

It has been reported that leaf callus tissues accumulate more Na^+ than leaves, as the Na^+ exclusion operative in whole plants was not expressed in cell culture (Garcia-Reina *et al.*, 1988b; Yang *et al.*, 1990). The results of this work corroborate this fact, as the differences between the Na^+ concentrations in leaves and calli from leaf were the highest in *L. esculentum* (where the exclusion mechanism is predominant) and intermediate in the hybrid (which showed a partial inclusion mechanism, although its accumulation capacity in the shoot was much lower than in *L. pennellii*, Figure 2). However, in the wild species, where the inclusion mechanism is predominant, no differences were found. This fact could explain, at least partly, the lower salt tolerance, measured as growth relative to the control, of *L. esculentum* in calli than in plants, whereas in *L. pennellii*, either similar or higher salt tolerances were found in calli than in plants at 140 mM NaCl. Moreover, in both *L. esculentum* and *L. pennellii*, there were no significant differences between the Na^+ concentrations of the calli from the different explant sources, whereas there were significant differences between the root and shoot Na^+ concentrations.

Concentrations of K^+ and Na^+/K^+ ratio

Either no decrease or a small decrease in K^+ concentrations were found in both whole plants and callus tissues of *L. esculentum* (Figure 3), which suggests that the ability of this species to maintain potassium selectivity under saline conditions (Gorham *et al.*, 1985) is expressed at both levels of organization. On the other hand, the ability to substitute Na^+ for K^+, shown generally by the wild tomato species (Cuartero *et al.*, 1992) was also found in both whole plant and callus tissue of *L. pennellii*. A similar physiological response to that of *L. pennellii* appeared in the hybrid, as the K^+ concentrations decreased in the organs and the calli with increasing concentrations of NaCl, with the K^+ decreases being even higher in the calli of the hybrid than in the calli of *L. pennellii*, except in the leaf calli.

It has been reported that Na^+/K^+ ratios in calli could be used as indicators of whole plant salt-tolerance (Watad *et al.*, 1983; Yang *et al.*, 1990). However, in our study, the salt tolerant wild species had a higher Na^+/K^+ ratio than did the salt-sensitive cultivated species only at the whole plant level. In calli of three tomato genotypes grown at different NaCl levels, Garcia-Reina *et al.*, (1988b) found Na^+/K^+ ratios similar to those of the culture medium, whereas higher and lower ratios were found in somaclones and seedlings, respectively, than in calli at all NaCl levels.

Tal and Shannon (1983) reported similar Na^+/K^+ ratios in two tomato hybrids (*L. esculentum* × *L. pennellii*) and the wild species. In the interspecific hybrid used in this study, the Na^+/K^+ ratios in the organs were lower than in those of *L. pennellii*; however, there was a higher capacity of Na^+ translocation to the shoot in the hybrid than in *L. esculentum*, as the Na^+/K^+ ratios in stems and leaves of the hybrid were about twice as high as in the same organs of the *L. esculentum* .

We conclude that in both cultivated and wild tomato genotypes, similar physiological responses were found at both levels of organization, which suggests that callus culture provides the opportunity to investigate cellular responses separate from whole-plant responses. In the hybrid, the physiological response was intermediate to those of both parents at the whole plant level, whereas in calli the response was more similar to *L. pennellii*.

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REFERENCES

BOURGEAIS, P., GUERRIER, G. and STRULLU, D. G. (1987). Adaptation au NaCl de *Lycopersicon esculentum*: étude comparative des cultures de cals ou de parties terminales de tiges. *Canadian Journal of Botany*, **65**, 1989–97.

BOURGEAIS, P. and GUERRIER, G. (1992). Salt-responses in *Lycopersicon esculentum* calli and whole plants. *Journal of Plant Physiology*, **140,** 494–501.

CUARTERO, J., YEO, A. R. and FLOWERS, T. J. (1992). Selection of donors for salt-tolerance using physiological traits. *New Phytologist*, **121,** 63–9.

DAINES, R. and GOULD, A. (1985). The cellular basis of salt tolerance studies with tissue cultures of the halophytic grass *Distichlis spicata*. *Journal of Plant Physiology*, **119,** 269–80.

DRACUP, M. (1991). Increasing salt tolerance of plants through cell culture requires greater understanding of tolerance mechanisms. *Australian Journal of Plant Physiology*, **18,** 1–15.

GARCÍA-REÍNA, G., MORENO, V. and LUQUE, A. (1988a). Selection for NaCl tolerance in cell culture of three Canary Island tomato land races. I. Recovery of tolerant plantlets from NaCl-tolerant cell strains. *Journal of Plant Physiology*, **133,** 1–6.

GARCÍA-REÍNA, G., MORENO, V. and LUQUE, A. (1988b). Selection for NaCl tolerance in cell culture of three Canary Island tomato land races. II. Inorganic ion content in tolerant calli and somaclones. *Journal of Plant Physiology*, **133,** 7–11.

GIBBS, J., DRACUP, M., GREENWAY, H. and MCCOMB, J. A. (1989). Effects of high NaCl on growth, turgor and internal solutes of tobacco callus. *Journal of Plant Physiology*, **134,** 61–9.

GORHAM, J., WYN JONES, R. G. and MCDONNELL, E. (1985). Some mechanisms of salt tolerance in crop plants. *Plant and Soil*, **89,** 15–40.

GULATI, A. and JAIWAL, P. K. (1992). Comparative salt responses of callus cultures of *Vigna radiata* (L.) Wilczek to various osmotic and ionic stresses. *Journal of Plant Physiology*, **141,** 120–4.

MURASHIGE, T. and SKOOG, F. (1962). A revised medium for rapid growth and bioassays for tobacco tissue culture. *Plant Physiology*, **15,** 373–97.

PÉREZ-ALFOCEA, F., BOLARIN, M. C., CARO, M. and GUERRIER, G. (1992). Influence of NaCl on N-assimilation metabolism in calli from *Lycopersicon pennelli*, *L. esculentum* and their hybrid. *Plant Physiology. Life Science Advances*, **11,** 189–98.

PÉREZ-ALFOCEA, F., ESTAÑ, M. T., CARO, M. and BOLARIN, M. C. (1993). Response of tomato cultivars to salinity. *Plant and Soil*, **150,** 203–11.

SMITH, M. K. and MCCOMB, J. A. (1981). Use of callus cultures to detect NaCl tolerance in cultivars of three species of pasture legumes. *Australian Journal of Plant Physiology*, **8,** 437–42.

SUBBARAO, G. V., JOHANSEN, C., KUMAR RAO, J. V. D. K. and JANA, M. K. (1990). Salinity tolerance in F1 of pigeonpea and a tolerant wild relative. *Crop Science*, **30,** 785–8.

TAL, M. (1984). Physiological genetics of salt resistance in higher plants: Studies on the level of the whole plant and isolated organs, tissues and cells. In: *Salinity tolerance in plants*. (Staples, R. C. and Toenniessen, G. H., Eds). New York: John Wiley & Sons, 301–20.

TAL, M., HEIKIN, H. and DEHAN, K. (1978). Salt tolerance in the wild relatives of the cultivated tomato: responses of callus tissues of *Lycopersicon esculentum* and *Solanum pennellii* to high salinity. *Zeitschrift für Pflanzenphysiology*, **86,** 231–40.

TAL, M. and SHANNON, M. C. (1983). Salt tolerance in the wild relative of the cultivated tomato: responses of *Lycopersicon esculentum*, *L. cheesmanii*, *L. peruvianum*, *Solanum pennellii* and F1 hybrids to high salinity. *Australian Journal of Plant Physiology*, **10,** 109–17.

VAN DEN BULK, R. W., LÖFFLER, H. J. M., LINDHOUT, W. H. and KOORNNEEF, M. (1990). Somaclonal variation in tomato: effect of explant source and a comparison with chemical mutagenesis. *Theoretical and Applied Genetics*, **80,** 817–25.

WATAD, A. A., REINHOLD, L. and LERNER, H. R. (1983). Comparison between a stable NaCl-selected *Nicotiana* cell line and the wild type. *Plant Physiology*, **73,** 624–9.

YANG, Y. W., NEWTON, R. J. and MILLER, F. R. (1990). Salinity tolerance in *Sorghum*. II. Culture response to sodium chloride in *S. bicolor* and *S. halepense*. *Crop Science*, **30,** 781–5.

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The yield and quality of ginger produced by micropropagated plants as compared with conventionally propagated plants

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SUMMARY

Meristem-derived 'Wynad local' ginger plants were micropropagated *in vitro*. The performance of micropropagated plants (MP) in the field, as compared with conventionally grown plants (CP), was evaluated from rhizome yield and other quality factors. 'Wynad local' ginger is usually harvested after eight months and MP ginger rhizomes harvested at the same time were comparable qualitatively and quantitatively to CP in the composition of starch, ash, acetone extract and volatile extract but only qualitatively similar in total oleoresin content, flavour profile, TLC aromagram and GC analysis, with less of each component than with CP ginger. The MP took two months longer cultivation to yield as much oleoresin and gingerols as in CP ginger. This difference was attributed to the fact that the micropropagated plants lacked a rhizome when planted and underwent shock during acclimatization. Periodic fluctuations in the yield and composition of various compounds are described.

THE benefits of clonal propagation using tissue culture techniques are well known, and the number of reports on micropropagating different species is ever increasing. *In vitro* micropropagation of ginger using meristem explants has been reported (Bhagyalakshmi and Singh, 1988; Noguchi *et al.*, 1988; Ikeda and Tanabe, 1989). In ginger, (*Zingiber officinale* Rosc.) the rhizome is the final product yielding pungent and flavour compounds used in food and pharmaceutical preparations. As the rhizome is also used for conventional propagation ginger, but is completely lacking in micropropagated ginger plants at planting, this poses many questions related to yield and durations of cultivation. Hence a study was undertaken to compare micropropagated ginger with that conventionally grown.

Pungency and flavour of ginger have long been recognized as important quality factors (Chen *et al.*, 1986; Govindarajan, 1982a). The oleoresin residue from solvent extraction of dried rhizomes contains the compounds responsible for flavour and pungency (Connell and Sutherland, 1969). The total oleoresin yield and other quality characters were examined as a test of the maturity of ginger.

MATERIALS AND METHODS

Meristem culture and shoot multiplication

The high oleoresin yielding cv. Wynad local was used throughout the experiment. Meristem tips from aseptically grown sword shoots were cultured on three-quarter strength Muashige and Skoog (1962) basal medium containing sucrose (6%), coconut milk (20%), ascorbic acid (100 mg l^{-1}), glutamine (400 mg l^{-1}), indole butyric acid (0.4 mg l^{-1}) and agar (0.8%). The conditions for rapid multiplication were standardized (Bhagyalakshmi and Singh, 1988) and the shoots were multiplied on the same medium used for meristem-tip culture but without glutamine, reduced sucrose (3%) and increased levels of 6-benzylaminopurine (4–5 mg l^{-1}). Shoots 6–9 cms tall with 2–3 good roots per shoot were selected for transplanting to soil. Short and rootless shoots were trimmed and transferred to fresh medium for further multiplication.