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# Relationship between vegetative and rhizome characters and final rhizome yield in micropropagated ginger plants (*Zingiber officinale* Rosc.) over two generations

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ABSTRACT

Correlation and path analysis for yield and yield contributing characters in two types of micropropagated ginger plants (plantlets directly regenerated from aerial stem explants and plantlets regenerated from aerial stem derived callus) were carried out over first and second generations in two varieties viz. var. 'Jamaica' and var. 'Varada'. Irrespective of the regeneration method, the *in vitro* derived plants showed high positive correlation and maximum positive direct effect of circumference of cormlets, length of cormlets and number of cormlets with the rhizome yield in the first generation. But tiller number exhibited negative correlation and negative direct effect with the rhizome yield in the first generation. In the second generation of the aerial stem regenerated plants, tiller number, number of nodes per cormlets, circumference of cormlets, number of cormlets and plant height exhibited high positive correlation and maximum direct effect with rhizome yield. However, in the second generation also, the callus regenerated plants showed the same trend as in the first generation. Even though the tiller number showed positive significant correlation with rhizome yield, it showed negative direct effect with the yield.



## 1. Introduction

Ginger (*Zingiber officinale* Rosc.) is a herbaceous, rhizomatous, perennial, the underground rhizome of which forms one of the important spices and medicines used by people all over the world.

In the absence of seed setting, biotechnological tools are now pressed into the service of improving ginger. Though variability, character association and path analysis are reported in the conventionally propagated ginger (Mohanty and Sarma, 1979; Sasikumar et al., 1992; Das et al., 1999; Singh, 2001; Abraham and Latha, 2003) no reports are there on these aspects of *in vitro* raised ginger though there are reports on *in vitro* propagation of ginger (Bhagyalakshmi and Singh, 1988; Balachandran et al., 1990; Nirmal Babu et al., 1992). Correlation and path analysis using *in vitro* regenerated plants are very relevant now since *in vitro* techniques are being a viable option in crop improvement of ginger.

The aim of the present study is to determine character association and its direct and indirect effects on rhizome yield of *in vitro* derived ginger plants over two different generations.

## 2. Materials and methods

Two ginger genotypes, var. 'Jamaica' and var. 'Varada' were used in the present study. Two groups of hardened micropropagated plants—plantlets regenerated directly from aerial stem (pseudostem = tiller) explants and plantlets regenerated from callus along with control were evaluated for yield in Completely Randomized Design (CRD) during the years 2003–2006 at the Indian Institute of Spices Research, Calicut, Kerala, India.

*In planta* aerial stem explants (1.0–1.5 cm—taken from the middle portion of the tiller containing apical meristem) cultured on half strength MS medium (Murashige and Skoog, 1962) supplemented with TDZ:IBA (1.0:1.0 mg $l^{-1}$) were used for direct plantlet regeneration. Callus induced from the *in planta* aerial stem explants, cultured on half strength MS medium containing 2,4-D (2 mg $l^{-1}$), were regenerated on the medium containing BAP and NAA (1.0:1.0 and 2.0:0.5 mg $l^{-1}$). Hardened *in vitro* derived plantlets were established in the field and evaluated every year. Planting of the tissue cultured plants was done under protected condition, in an experimental shed of 24 m × 6 m size with 50% shade, in polythene bags (30 cm × 40 cm) containing a mixture (approximately 4 kg) of garden soil, sand, coir dust and cow dung (2:1:1:1) + 10 g *Trichoderma* (CFU = $10^{14}$ g). Normal manuaring (organic) and cultural practices were adopted.

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Observations were taken on plant height, number of tillers, number of leaves, leaf length, leaf width, number of cormlets per rhizome (total number of distinguishable rhizome branches present in a whole clump), length and circumference of cormlets, internodes per cormlet, internodal length and weight of rhizome from 10 randomly selected plants of each group. The aerial morphological characters were recorded at the maximum vegetative growth stage (6 months old) and the rhizome characters after harvest. The pooled data on yield and yield attributes for the different years were subjected to correlation and path analysis (Dewey and Lu, 1959; Singh and Chaudhary, 1985).

For the evaluation of tissue cultured ginger plants in the second generation, rhizomes obtained from the first generation plants were planted in the experimental shed and observations were taken as described above and analyzed.

## 3. Results and discussions

### 3.1. Aerial stem regenerated plants

Correlation analysis of the aerial stem regenerated plants revealed a high, positive and significant correlation of fresh rhizome yield per plant with circumference of cormlets (0.92) followed by length of cormlets (0.87) and number of cormlets (0.76). Plant height also had a positive significant correlation with number of leaves, leaf length, leaf width, length of cormlets, circumference of cormlets, internodal length and rhizome yield. Tiller number exhibited negative correlation with all the characters studied except leaf number. The characters like tiller number (−0.8) and number of leaves (−0.03) showed negative correlation with the rhizome yield (Table 1).

In the second generation, a positive significant relationship of rhizome yield with tiller number (0.50) and number of nodes per cormlets (0.27) was observed. Plant height showed positive but non-significant correlation with the yield (0.04) and leaf number (0.16). However, plant height showed a positive significant correlation with leaf length (0.57) and circumference of cormlets (0.50). Tiller number exhibited positive but non-significant correlation with plant height unlike in the first generation. High negative significant associations between yield and length of cormlets (−0.71) as well as leaf length (−0.52) was observed in the second generation, a reverse trend as observed in the first generation (Table 1).

Partitioning of the correlation coefficients into direct and indirect effects revealed that circumference of cormlets followed by length of cormlets and number of cormlets exhibited maximum positive direct effect on rhizome yield in the first generation. Maximum negative direct effect on rhizome yield was exhibited by tiller number. Circumference of cormlets through length of cormlets followed by number of cormlets, internodal length of cormlets and leaf width exhibited maximum positive indirect effects. Maximum negative indirect effect was recorded by circumference of cormlets through tiller number and length of cormlets through tiller number. All other effects were negligible. A residual effect of 0.22 indicated that the characters studied accounted for most of the variability in the first generation. However, the residual effect in the second generation was 0.48.

In the second generation, the characters such as plant height followed by number of nodes per cormlet and leaf width showed high positive direct effect with yield. Leaf length and length of cormlets exhibited high negative direct effect with rhizome yield. All other direct effects were negligible. Plant height through leaf length followed by length of cormlets through tiller number and leaf length through number of cormlets showed good positive indirect effects. Maximum negative indirect effect on fresh rhizome yield was observed in case of leaf length through plant height followed by leaf length through length of cormlets and length of cormlets through leaf length.

### 3.2. Callus regenerated plants

High positive significant relationship of rhizome yield with circumference of cormlets (0.96) followed by cormlet length (0.93) and number of cormlets (0.92) was observed. Leaf width, number of nodes per cormlets, internodal length, plant height, leaf number and leaf length also exhibited positive significant association with rhizome yield. Plant height exhibited significant positive correlation with all the characters except tiller number, whereas number of tillers showed negative non-significant correlation with rhizome yield and all other characters studied (Table 2).

**Table 1**
Correlation matrix for morphological and yield characters in aerial stem regenerated plants (first and second generations)

| Character | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | X 10 | Y 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| X 1 | 1.00 | −0.25 | 0.32 | 0.83** | 0.37* | 0.17 | 0.41* | 0.34* | −0.26 | 0.32 | 0.48** |
| X 1 (S) | 1.00 | 0.11 | 0.16 | 0.57** | 0.06 | −0.37* | 0.17 | 0.50** | −0.10 | 0.18 | 0.04 |
| X 2 | | 1.00 | 0.03 | −0.07 | −0.59** | −0.57** | −0.74** | −0.71** | −0.44** | −0.42* | −0.80** |
| X 2 (S) | | 1.00 | −0.16 | −0.23 | 0.10 | −0.07 | −0.50** | 0.19 | 0.15 | 0.11 | 0.50** |
| X 3 | | | 1.00 | 0.33* | −0.23 | −0.12 | 0.00 | −0.11 | −0.20 | −0.05 | −0.03 |
| X 3 (S) | | | 1.00 | 0.33* | 0.11 | −0.13 | 0.25 | 0.24 | 0.22 | 0.24 | −0.15 |
| X 4 | | | | 1.00 | 0.39* | 0.10 | 0.24 | 0.34* | −0.28 | 0.45** | 0.39* |
| X 4 (S) | | | | 1.00 | 0.34* | −0.45** | 0.44** | 0.29 | −0.10 | 0.28 | −0.52** |
| X 5 | | | | | 1.00 | 0.54** | 0.57** | 0.73** | 0.12 | 0.71** | 0.75** |
| X 5 (S) | | | | | 1.00 | −0.10 | −0.16 | 0.14 | 0.02 | 0.14 | 0.04 |
| X 6 | | | | | | 1.00 | 0.59** | 0.78** | 0.30 | 0.49** | 0.76** |
| X 6 (S) | | | | | | 1.00 | −0.42** | −0.06 | −0.20 | 0.28 | 0.24 |
| X 7 | | | | | | | 1.00 | 0.79** | 0.61** | 0.52** | 0.87** |
| X 7 (S) | | | | | | | 1.00 | −0.23 | −0.12 | 0.05 | −0.71** |
| X 8 | | | | | | | | 1.00 | 0.45** | 0.76** | 0.92** |
| X 8 (S) | | | | | | | | 1.00 | 0.05 | −0.04 | 0.24 |
| X 9 | | | | | | | | | 1.00 | 0.15 | 0.40* |
| X 9 (S) | | | | | | | | | 1.00 | 0.20 | 0.27 |
| X 10 | | | | | | | | | | 1.00 | 0.64** |
| X 10 (S) | | | | | | | | | | 1.00 | −0.20 |

*Significant at 0.05 level. **Significant at 0.01 level ($N$ = 40). Plant height (X 1), number of tillers (X 2), number of leaves (X 3), leaf length (X 4), leaf width (X 5), number of cormlets (X 6), length of cormlets (X 7), circumference of cormlets (X 8), number of nodes per cormlets (X 9), length of internodes (X 10) and weight of rhizomes (Y 1). X 1(S)–X 10 (S): characters of second generation.

**Table 2**
Correlation matrix for morphological and yield characters in callus regenerated plants (first and second generations)

| Character | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | X 10 | Y 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| X 1 | 1.00 | −0.08 | 0.68** | 0.65** | 0.63** | 0.58** | 0.72** | 0.68** | 0.38* | 0.63** | 0.70** |
| X 1 (S) | 1.00 | 0.73** | 0.60** | 0.75** | 0.72** | 0.67** | 0.84** | 0.80** | 0.68** | 0.45** | 0.85** |
| X 2 | | 1.00 | −0.09 | −0.10 | −0.20 | −0.22 | −0.23 | −0.21 | -0.17 | −0.08 | −0.24 |
| X 2 (S) | | 1.00 | 0.47** | 0.46** | 0.66** | 0.61** | 0.70** | 0.73** | 0.59** | 0.44** | 0.69** |
| X 3 | | | 1.00 | 0.59** | 0.55** | 0.60** | 0.67** | 0.66** | 0.57** | 0.56** | 0.67** |
| X 3 (S) | | | 1.00 | 0.51** | 0.64** | 0.50** | 0.66** | 0.68** | 0.35* | 0.08 | 0.61** |
| X 4 | | | | 1.00 | 0.68** | 0.46** | 0.57** | 0.59** | 0.26 | 0.59** | 0.65** |
| X 4 (S) | | | | 1.00 | 0.46** | 0.37* | 0.46** | 0.66** | 0.36* | 0.27 | 0.55** |
| X 5 | | | | | 1.00 | 0.77** | 0.77** | 0.85** | 0.66** | 0.71** | 0.87** |
| X 5 (S) | | | | | 1.00 | 0.66** | 0.67** | 0.79** | 0.49** | 0.37* | 0.69** |
| X 6 | | | | | | 1.00 | 0.83** | 0.95** | 0.82** | 0.70** | 0.92** |
| X 6 (S) | | | | | | 1.00 | 0.78** | 0.84** | 0.72** | 0.36* | 0.84** |
| X 7 | | | | | | | 1.00 | 0.88** | 0.81** | 0.61** | 0.93** |
| X 7 (S) | | | | | | | 1.00 | 0.86** | 0.82** | 0.31 | 0.89** |
| X 8 | | | | | | | | 1.00 | 0.80** | 0.76** | 0.96** |
| X 8 (S) | | | | | | | | 1.00 | 0.72** | 0.42* | 0.90** |
| X 9 | | | | | | | | | 1.00 | 0.43* | 0.77** |
| X 9 (S) | | | | | | | | | 1.00 | 0.43* | 0.76** |
| X 10 | | | | | | | | | | 1.00 | 0.73** |
| X 10 (S) | | | | | | | | | | 1.00 | 0.50** |

*Significant at 0.05 level. **Significant at 0.01 level ($N = 40$). Plant height (X 1), number of tillers (X 2), number of leaves (X 3), leaf length (X 4), leaf width (X 5), number of cormlets (X 6), length of cormlets (X 7), circumference of cormlets (X 8), number of nodes per cormlets (X 9), length of internodes (X 10) and weight of rhizomes (Y 1). X 1(S)–X 10 (S): characters for second generation.

Association analysis in the second generation revealed that circumference of cormlets (0.90) showed maximum positive significant correlation with yield followed by length of cormlets (0.89), plant height (0.85) and number of cormlets (0.84). In the first generation too circumference of cormlets followed by length of cormlets and number of cormlets showed positive significant association with rhizome yield. Plant height and tiller number showed positive significant correlation with all the characters studied (Table 2).

Partitioning of the correlation coefficients into direct and indirect effects revealed that length of cormlets followed by circumference of cormlets and number of cormlets exhibited maximum direct effect on rhizome yield during the first generation. The same trend was observed in the second generation too. Plant height, number of nodes per cormlets, internodal length and tiller number exhibited negative direct effect on rhizome yield in the first generation. However, in the second generation plant height exhibited positive direct effect with rhizome yield. Length of cormlets showed good indirect effect through most of the characters studied in both the generations. Plant height through leaf length followed by length of cormlets exhibited maximum negative indirect effect with rhizome yield in the first generation whereas number of nodes per cormlets through length of cormlets showed maximum negative indirect effect followed by number of nodes per cormlets through circumference of cormlets in the second generation. Residual effects of 0.16 and 0.29 during the first and second generations, respectively, indicated that most of the variability is accounted by the characters studied.

Earlier reports on character association in ginger revealed that yield contributing traits like plant height, tiller number, leaf number, width of leaves, rhizome width, rhizome length and thickness of secondary rhizome exhibited maximum positive correlation and maximum positive direct effect with rhizome yield in conventionally propagated plants (Mohanty and Sarma, 1979; Sasikumar et al., 1992; Pandey and Dhobal, 1993; Das et al., 1999; Abraham and Latha, 2003).

Character association study in micropropagated ginger is not reported in literature. In the present study, irrespective of the regeneration methods, circumference of cormlets, length of cormlets and number of cormlets showed high positive correlation and maximum positive direct effect with rhizome yield in the first generation. But tiller number exhibited negative correlation and negative direct effect with the rhizome yield in the first generation. However, in the second generation of the aerial stem regenerated plants, tiller number, number of nodes per cormlets, circumference of cormlets, number of cormlets and plant height exhibited high positive correlation and maximum direct effect with rhizome yield as observed in the conventionally propagated ginger plants (Sasikumar et al., 1992; Pandey and Dhobal, 1993; Das et al., 1999; Abraham and Latha, 2003). Ratnambal et al. (1980) reported that when number of tiller increased, the yield also increased correspondingly in the conventionally propagated plants. Thus these results indicate that the aerial stem regenerated plants tends to exhibit similarity with the conventionally propagated plants from the second generation itself. However, in the second generation also, the callus regenerated plants showed the same result as in the first generation. Even though tiller number showed positive significant correlation with rhizome yield in the callus derived population, it showed negative direct effect with yield suggesting that the callus regenerated plants take at least three crop seasons to stabilize, probably due to the inherent instability of the unorganized tissue (callus).

A comparison of the fresh rhizome yield of two varieties of *in vitro* raised ginger with conventionally propagated plants revealed that direct regenerated plants are either better or comparable in yield with conventionally propagated plants by the second generation itself, though there was varietal variation (Table 3). However, in both the generations the callus regenerated plants turned out to be poor performers.

Comparatively low yield of micropropagated (callus derived) as compared to conventionally propagated plants over first and second generations was reported earlier (Samsudeen, 1996). Similar trend in yield of micropropagated and conventionally propagated ginger from subtropical Queensland was also observed (Smith and Hamill, 1996). Bhagyalakshmi and Singh (1988) reported that vegetative bud regenerated plants were at par with the conventionally propagated ones except that they need longer (additional 2 months) crop duration for the same effect.

The present study thus implies that direct regenerated ginger plants from aerial stem explants would behave more or less same

**Table 3**
Comparative rhizome yield of micropropagated and conventionally propagated ginger plants over two generations

| Generation | Weight of rhizome/plant (g) | | | | | |
|---|---|---|---|---|---|---|
| | Aerial stem regenerated plant | | Callus regenerated plant | | Conventionally propagated plant | |
| | 'Jamaica' | 'Varada' | 'Jamaica' | 'Varada' | 'Jamaica' | 'Varada' |
| First | 54.7 | 42.0 | 16.1 | 12.9 | 77.2 | 91.3 |
| Second | 157.0 | 76.4 | 28.1 | 24.2 | 84.1 | 96.8 |

as the conventionally propagated plants by the second generation. Thus, *in vitro* improvement of ginger using this method would be more apt than going for callus regeneration protocol especially for transformation and other biotechnological approaches involving field evaluation and selection. The important yield contributing aerial characters that can be useful for selecting the high yielding population will be tiller number and plant height.

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