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Developmental Variation among Improved Coffee Hybrids Propagated through Somatic Embryogenesis

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Abstract A study was conducted to assess the response of improved coffee varieties to somatic embryogenesis and identify varieties that can be included in multiplication programme using this technique. Young fully expanded leaves from six varieties N39-1, N39-5, KP423-1, KP423-3, CVT1-2 and CVT2-1 planted at Lyamungu were surface sterilized for 30 minutes under agitation using calcium hypochlorite solution, cut in small explants approximately 1 cm^2. Seven explants each were plated in 5 magenta jars (6.5cm diameter) per variety, cultured in Murashige Skoog medium with initiation additives (MS1) for 6 weeks, and embryonic callus development additives (MS2) for 6 months. The time required for callus induction was observed during the first six weeks. Callus formation continued to be monitored up to six months. Then callus weights were taken per jar and results expressed as percentage of the established average weight of calli per genotype, and were routinely managed afterwards. Each magenta jar was treated as a replication, allowing for RCD design, and individual weights were exposed to ANOVA using STAT statistical software. The results showed some difference in both callus formation time and callus weight among the genotypes tested, the latter being significant at $P<0.05$. Explants from varieties CVT1-2 and CVT2-1 were fastest developing (3 weeks) followed by KP423-3 and KP423-1 (4 weeks) while N39-1 was slowest (5 weeks). The highest mean weight and percentage of callus development was observed in explants obtained from variety KP423-3 (86.25%), KP423-1 (83.73%) followed by N39-3 (63.75%) and CVT1-2 (61.25%), while the least performers were N39-1 (46.25%) and CVT2-1 (43.75%). This study has shown that response to somatic embryogenesis differs with varieties, opening up avenue for future screening of the remaining 13 varieties. Varieties KP423-3 and KP423-1, with high percent callus per explant and average initiation time, are hereby recommended as pioneers for investors interested in massive somatic embryogenesis of Arabica coffee in Tanzania.

Keywords: *coffee hybrids, propagation, somatic embryogenesis*

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1. Introduction

Somatic embryogenesis is the type of tissue culture whereby single or groups of cells initiate the development pathway that leads to reproducible regeneration of non-zygotic embryos capable of germinating to form complete plants [1]. Its advantages over the conventional vegetative propagation include small space required, high multiplication rate and a controlled culture condition free from both seasonal dependence and microbes. It has been used successfully in multiplication of various crops [2]. In the case of coffee, one of the most important crops, it can allow a rapid propagation of selected clones of F1 hybrids, thereby avoiding manual hybrid seeds production and cuttings which are costly and difficult for Arabica [3,4].

One of the long-standing challenges facing the coffee sub-sector in Tanzania have been diseases, notably coffee berry disease (CBD) incited by a fungus *Colletotrichum kahawae* Waller & Bridge; and leaf rust (CLR) incited by another fungus *Hemileia vastatrix* Berk et Br [5]. The two diseases can cause yield loss ranging from 20-60% and their control cost, in terms of fungicides, amounts to 30-50% of the total cost of production in Tanzania [6]. Hence the most important priority unanimously identified by stakeholders is the development and distribution of varieties resistant to these two diseases. Tanzania Coffee Research Institute (TaCRI) mounted a meticulous breeding programme and has so far developed 19 Arabica hybrids that, in addition to resistance to those diseases, combine high yields, large bean size and good cup quality [7]. The entire coffee area countrywide is estimated at 265,000 ha, of which 53,000 ha (about 20%) has so far been covered by improved varieties, mainly through cutting and grafting. The old coffee land that still needs new improved seedlings was estimated by [8] as 212,000 ha which, at the density of 2,000 trees per ha recommended by TaCRI for tall varieties, gives a requirement of 424 million seedlings.

In addition to TaCRI substations, district and farmer group nurseries that produce clonal, grafted and manually

hybridized seedlings, we are encouraging serious investors with appropriate facilities to pursue somatic embryogenesis as a complementary approach [9]. The objective of this work was to determine the variation in somatic embryogenesis performance of some Tanzanian improved Arabica coffee F1 hybrids, so as to identify best performers that we can recommend as pioneers for the potential investors to start with.

2. Materials and Methods

Leaf samples from six improved *Coffea arabica* L. varieties (KP423-1, KP423-3, N39-1, N39-3, CVT1-2 and CVT2-1) were collected from the field at TaCRI Lyamungu. These samples were washed with tap water then rinsed with distilled water and later dipped for 30 seconds in a 70% ethanol solution. Then they were surface sterilized for 30 minutes under agitation in a solution of calcium hypochlorite ($CaCl_2O_2$) at 40 g l^{-1} and finally rinsed three times in sterile distilled water. The leaves were cut in small explants approximately ($1cm^2$) avoiding mid veins, margins, apical and basal portions. A glass beads sterilizer maintained at 250°C and flame were used frequently to sterilize the tools during manipulation. Seven explants were plated per magenta jar (6.5cm diameter) containing 20 mls of MS1 media [10] (see Appendix 1), such that the upper epidermis were in contact with the medium. Each variety was cultured in 5 magenta jars, each one having 7 explants.

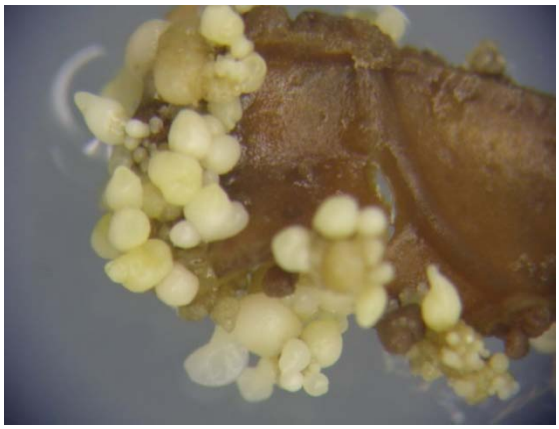

Figure 1. Embryos at sub-culturing stage

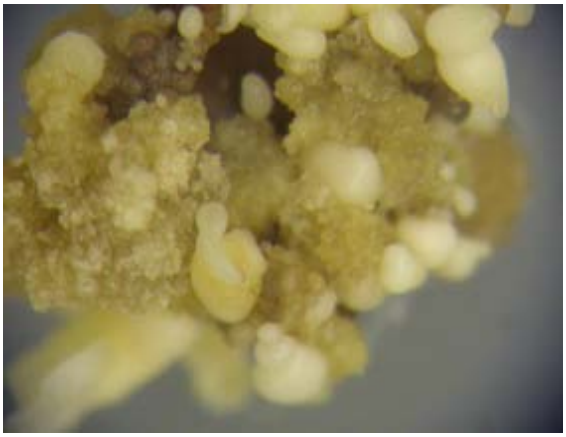

Figure 2. Embryos ready for development of plantlets

Figure 3. Germinated embryos

Figure 4. Plantlets at acclimatization stage

During the first six weeks the time required for callus development was observed. After four to six weeks in the callus induction medium, the explants were sub cultured into the advanced callus induction containing MS2 media (see Appendix 1) based on [11], and [12]. The cultures were incubated in a dark condition at 25±1°C. Sub-culturing was done every month (Figure 1). During sub-culturing, the aqueous callus and contaminated jars were discarded. Evaluation of the amount of callus developed was done 6 months after initiation.

Data were recorded for the explant reactivity (embryogenic callus development time in weeks), and descriptively analyzed. In the latter case, calli were weighed per jar and results expressed as percentage of the established average weight of calli per genotype (0.698 g per 5 jars). A completely randomized design (CRD) was used for the evaluation of the six varieties and five magenta jars as replications as in [13,14]. Individual weights per variety and jar were subjected to analysis of variance using STAT software. $P < 0.05$ was considered statistically significant.

Embryogenic callus was carefully selected and placed in maintenance medium for multiplication and differentiation to torpedo shaped embryos. Embryos were transferred to DES1 media for germination and development to plantlets (Figure 2) as shown in Appendix 1. Finally, plantlets were hardened and transplanted to the green house for acclimatization (Figure 3 and Figure 4).

3. Results and Discussion

Three weeks after incubation on callus induction medium, the coffee leaf tissues started to proliferate at the incision site of the leaf on two varieties i.e. CVT1-2 and CVT2-1. The forth week after initiation, three varieties

(KP423-3, KP423-1 and N39-1) proliferated callus. The least active variety was N39-1 that produced callus at the fifth week after initiation (Figure 5). The five-week period was also observed by [15] in their work on the protoplast from the shoot apices of pea plants.

Average weights of embryogenic callus five months after initiation are shown in Table 1, together with their standard deviations and standard error of the means. Two varieties KP423-3 and KP423-1 produced the highest amount of 0.602 and 0.584 g callus respectively, followed by N39-3 and CVT1-2. The least performers were N39-2 and CVT2-1. The attempted ANOVA for varieties and jars (Table 2) indicated a significant variation ($p < 0.05$) among the varieties; and expectedly, no significant variation among the jars since the set conditions were the same.

Table 1. Mean callus weight for the five Magenta jars

Variety	Mean wt (g)	Standard deviation	Standard error of the mean
KP423-3	0.6020	0.00255	0.00114
KP423-1	0.5840	0.00490	0.00219
N39-3	0.4450	0.04031	0.01803
CVT1-2	0.4270	0.01911	0.00854
N39-1	0.3228	0.00239	0.00107
CVT2-1	0.3050	0.00381	0.00170

Table 2. ANOVA for varieties and Magenta jars

Source of Variation		SS	Df	MS	F	Sig
Variety	Between groups	87	26	3.346	20.077	0.015*
	within groups	0.5	3	0.167		
	Total	87.5	29			
Jar	Between groups	49	26	1.885	0.514	0.853ns
	within groups	11	3	3.667		
	Total	60	29			

Percentage of calli formed varied according to variety; the lowest callus percentage obtained from CVT 2-1 (43.75%) and N39-1 (46.25%) followed by N39-3 and CVT1-2. On the other hand, the highest percentage were obtained from variety KP423-3 and KP423-1 with respective percentages of 86.25% and 83.75% (Figure 6). These upper figures are close to 85% observed by [16] in their work on a medicinal plant *Cleome rosea*. Similar trends were also observed by [17] in their pilot work on Tanzanian improved coffee genotypes. This implies that, if one needed to produce more seedlings per explant, the varieties of choice would be KP423-3 and KP423-1.

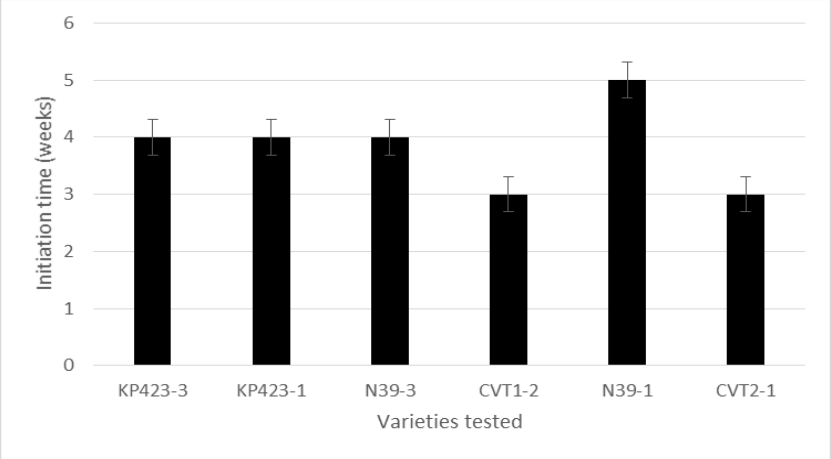

Figure 5. Varietal difference in callus induction period

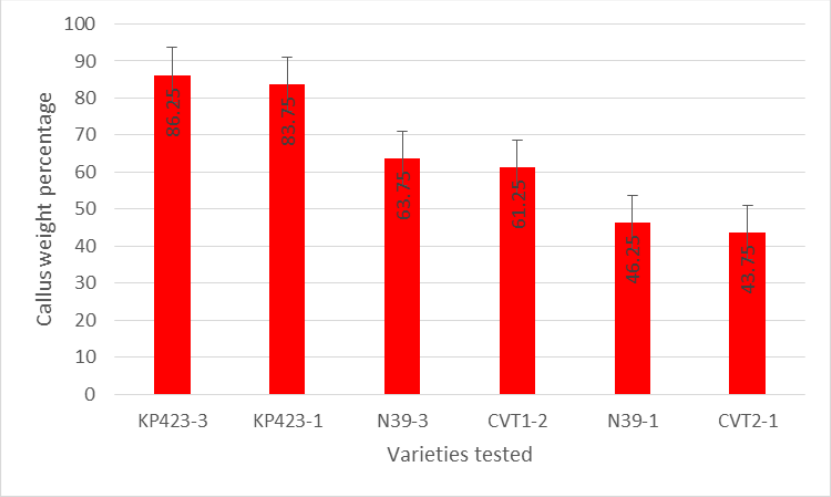

Figure 6. Varietal difference in callus weight percentage

Callus formation is an important initial phase of somatic embryogenesis [20]. It has been studied by many researchers at different times and for different crops. The study by [19] on its induction and repression mechanisms, associated the process with the action of growth hormones auxin and cytokinin, a theory also supported by [2].

Other factors documented to influence callus formation are media composition, exposure to light and incubation temperature [20,21]. However, virtually all the incubation conditions were made constant in this work, leaving genomic attributes, as noted by [22], as the sole source of variation. Further research is envisaged to establish the genomic attributes responsible for this in coffee.

Regardless of the plant species, the various somatic embryogenesis protocols generally follow a similar process flow: a) induction of embryogenic calli followed by their identification and selection by physical isolation; b) multiplication of the embryogenic cells; c) regeneration of large numbers of embryos from these cells; d) conversion to mature embryos able to regenerate plants. A number of previous studies like [16,18] and [21] covered both callus formation and plant regeneration. From the pilot study on coffee, [17] noted that callus formation is a determinant phase to the ultimate number of seedlings per explant. For this reason, Stage 3 was performed routinely and was not made part of this work.

Comparing the relative advantage of early callus initiation versus that of more calli per explant, the latter outweighs the former in a Tanzanian context whereby the interest is more seedlings. And because the higher yielding varieties KP423-3 and KP423-1 did not perform too badly in initiation time (4 weeks), they are likely to be the varieties of choice.

4. Conclusion

In this study, six of the 19 improved hybrid coffee varieties with good attributes in terms of diseases resistance, high yields and good cup quality, were screened for their reaction to somatic embryogenesis so as to identify pioneers to be included in the programme of large-scale propagation of seedlings. While varieties CVT1-2 and CVT2-1 initiated callus earlier, varieties KP 423-3 and KP 423-1 developed more calli per explant differing significantly from the rest. The two were also average in callus initiation time. This study has shown that response to somatic embryogenesis differs with varieties, opening up avenue for future screening of the remaining 13 varieties. Based on these findings, we recommend varieties KP423-3 and KP423-1 as pioneers for investors who will be interested in massive somatic embryogenesis for Arabica coffee in Tanzania.

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References

[1] Sharma, G., Jagetiya, S. and Dashora, R. (2015). General techniques of plant tissue culture. Lulu Press Inc., Raleigh, North Carolina, USA. 28pp.

[2] Suman, S. (2017). Plant tissue culture: A promising tool of quality material production with special reference to micropropagation of banana. Biochemical Cell Architecture Vol. 17 (1): 1-26.

[3] Berthouly M and Michaux-Ferriere N. (1996) High frequency somatic embryogenesis *Coffea canephora*: Induction conditions and histological evolution. Plant Cell, Tissue and Organ culture, 44:169-176.

[4] Berthouly, M and Etienne, H. (1999) Somatic embryogenesis of coffee In: Somatic Embryogenesis in Woody Plants. Jain, S.M, Gupta P.K and Newton, R.J (Eds), Kluwer Academic Publishers, Dordrecht The Netherlands pp 259-287.

[5] Teri, J.M., Kilambo, D.L., Mtenga, D.J., Nyange, N.E., Nzalawahe, T.S., Chipungahelo, G.S., Kipokola, T.P. and Kullaya, I.K (2004). Improved Arabica varieties for the benefit of Tanzania coffee growers. In *20th International Conference on Coffee Science*. Bangalore, India, pp. 1187-1191.

[6] Mtenga, D.J., Reuben, S.O., Varzea, V.M., Pereira, P., Kilambo, D.L. and Teri, J.M. (2008). Variation in resistance to coffee berry disease (Colletotrichum kahawae) among germplasm progenitors in Tanzania. In *22nd International Conference on Coffee Science*. Campinas, Brazil. 7pp.

[7] Kilambo, D.L., Mtenga, D.J., Ng'homa, N.M., Ngomuo, R., Teri, J.M. and Mlwilo, B. (2014). A decade of contributing to a profitable and sustainable coffee industry in Tanzania: The Arabica and Robusta improvement programmes. In *25th International Conference on Coffee Science*. Armenia, Colombia: 42-46.

[8] Diligent Consulting (2017). Strategic Action Plan IV. 2017/2018 - 22/2023 For Tanzania Coffee Research Institute: Prepared with financial support from EDF, DSM: 49pp.

[9] Mbwambo, S.G., Maro, G.P. and Nkya, E.O. (2018). Effects of seedling multiplication methods on growth and yield of new tall and compact Coffea Arabica varieties in Tanzania. In *27th International Conference on Coffee Science*. Portland, Oregon, USA.

[10] Murashige T. and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473-497.

[11] Van Boxtel, J. and Berthouly, M. (1996). High Frequency somatic embryogenesis from coffee leaves: Factors influencing embryogenesis and subsequent proliferation and generation in liquid medium. Plant Cell, Tissue and Organ Culture, 44: 7-17.

[12] Etienne, H. (2005). Somatic embryogenesis protocol: Coffee (*Coffea arabica* L. and C. P.). *In:* S.M. Jain and P.K. Gupta (Eds.). Protocol for Somatic Embryogenesis in Woody Plants. Springer, the Netherlands. pp 167-179.

[13] Kumlay, A.M. and Ercisi, S. (2015). Callus induction, shoot proliferation and root regeneration of potato (Solanum tuberosum L) stem node and leaf explants under long day conditions. Biotechnology and Biotechnological equipment Vol. 29 (6): 1075-1084.

[14] Reddy, N.V., Ghosh, S.B., Chandramouli, K., Vijaya, T., Pushpalatha, B., Dandu, A. and Pragathi, D. (2013). Optimization of biomass yield and asiaticoside accumulation in the callus cultures from the leaves of Cantella asiatica L. Urban. World Journal of Pharmacy and Pharmaceutical Siences Vol. 2 (4): 5966-5976.

[15] Gamborg, O.L., Shyluk, J. and Kartha, K.K. (1975). Factors affecting the isolation and callus formation in protoplast from the shoot apices of Pisum sativum, L. Plant Science Letters 4 (5): 285-292.

[16] Simoes, C., Albarello, N., Callado, C.H., de Castro, T.C. and MMansur, E. (2010). Somatic embryogenesis and plant regeneration from callus cultures of Cleome rosea Vahl. Brazilian Archives of Biology and Technology 53 (3). ISSN 1516-8913.

[17] Ngomuo, R., Mtenga, D. J.,Kilambo, D. L., Teri, J. M. (2012). Progress with somatic embryogenesis of improved Arabica and Robusta coffee in Tanzania. In: 24thInternational Conference on Coffee Science (ASIC) held 11th -16th November 2012 San Jose, Costa Rica.

[18] Polanco, M.C., Pelaez, M.I. and Ruiz, M.L. (1988). Factors affecting callus and shoot formation from in vitro cultures of Lens culinaris Medik. Plant cell, Tissue and Organ Culture 15: 175-182.

[19] Ikeuchi, M., Sugimoto, K. and Iwase, A. (2013). Plant callus: Mechanisms of induction and repression. Plant Cell 25 (9): 3159-3173.

[20] Zhao, D., Wang, Y. and Zhao, J. (1998). Effects of some factors on callus growth and flavonoids formation on callus cultures of Saussurea medusa. China Journal of Biotechnology 14 (3): 141-147.

[21] Benderradji, L., Brini, F., Kellon, K., Ykhlef, N., Djekoum, A., Masmondi, K. and Bouzerzour, H. (2011). Callus induction, proliferation and plantlet regeneration of two bread wheat genotypes under saline and heat stress conditions. International Scholarly Research Notice 2012: 8pp.

[22] Tuskan, G.A., Mewalal, R., Gunter, L.E., Palla, K.J., Carter, K., Jacobson, D.A., Jones, P.C., Garcia, B.J., Weighill, D.A., Hyatt, P.D., Yang, Y, Zhang, J., Reis, N., Chen, J.G. and Muchero, W. (2018). Defining the genetic component of callus formation: a GWAS approach. PLOS One 13 (8). 020519.

Appendix 1. Composition of the culturing media used

Name	Main component	Additives
MS1	Half strength of Murashige-Skoog medium	Thiamine (5.0 mg L^{-1}), myo-inositol (50 mg L^{-1}), nicotinic acid (0.5 mg L^{-1}), pyridoxine HCL (0.5 mg L^{-1}), 2iP (2.0 mg L^{-1}) casein hydrolysate (100 mg L^{-1}), malt extract (400 mg L^{-1}), 2,4-D (0.5 mg L^{-1}), AIB (1.0 mg L^{-1}), sucrose (30 g L^{-1}) and phytagel (2.5 g L^{-1}) as solidifier agent.
MS2	Half strength of Murashige-Skoog medium	Thiamine (20 mg L^{-1}), myo inositol (200 mg L^{-1}), casein hydrolysate (200 mg L^{-1}), malt extract (800 mg L^{-1}), 2, 4-D (1 mg L^{-1}), BAP (4 mg L^{-1}), adenine sulfate (60 mg L^{-1}), sucrose (30 g L^{-1}) and phytagel (2.5 g L^{-1}) as solidifier agent
DES1	Half strength of Murashige-Skoog medium	Myo- inositol (100 mg L^{-1}), nicotinic acid (1.0 mg L^{-1}), pyridoxine HCL (1.0 mg L^{-1}), thiamine HCL (1.0 mg L^{-1}), pantothenic acid (1.0 mg L^{-1}), biotin (0.01 mg L^{-1}), BAP (0.112 mg L^{-1}), sucrose (10 g L^{-1}) and phytagel (4.0 g L^{-1}).