



Early decapitation on African plum control-pollinated seedlings and consequences on subsequent growth in Cameroon

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Abstract

A decapitation test was used to assess control-pollinated seedlings' growth in *Dacryodes edulis*. Thirty nine weeks after sowing, pot-grown seedlings belonging to 13 control-pollinated progenies from 4 provenances were decapitated by removal the shoot apex or the uppermost node, leaving shoots of uniform height of 40 cm. The stem size (height and diameter) were measured and the number of leaves counted weekly for a period of 39 weeks after sowing (WAS) in the nursery. Then, the length and number of lateral shoots (twigs) subsequently formed were measured during a period of 2 to 8 weeks after decapitation (WAD) in a net house to determine the branching frequency. Previous to decapitation, seedling height did not showed significant variation ($P > 0.05$) in all crosses combined, 39 WAS. However, seedling collar diameter and number of leaves showed significant variation ($p < 0.05$). Eight weeks after decapitation, only the number of vigorous twigs showed significant variation ($p < 0.05$) in all crosses combined. The number of weeks after decapitation was the strong predictor of the shoot elongation. According to the conceptual basis of the decapitation test interpretation, the "Sprouting and the Dominance Phases" were not observed in the present study. In fact, *D. edulis* showed a synchronal shoot growth after decapitation. This habit could be used as a predictive test according to the relationship between branching habit and harvest index.

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Introduction

Most of the time, funds have been provided to improve major cereals (rice, maize and sorghum) and pulses like groundnut, cowpea, wheat and faba bean (Onwubiko *et al.*, 2011; Yahuza, 2012). More attention to improve and diversify crop production can help to reach food self-sufficiency and its use can allow overcoming protein malnutrition (FAO, 2009). One of the urgent actions to address this problem is the adoption of agroforestry practices (Tchoundjeu *et al.*, 2006; 2008) which lead to the integration in farmers systems of native species with high nutritional and commercial values across the exotic fruit trees grown extensively (Leipzig, 1996). Among these natives species, *Dacryodes edulis* (G.Don.) H.J.Lam.) or African plum, an oleaginous fruit tree belonging to the family Burseraceae in the Gulf of Guinea (Bourdeaut, 1971), is one of a number of indigenous fruits under domestication (Tchoundjeu *et al.*, 2002; 2008) and widely commercialized in Cameroon, Gabon, Democratic Republic of Congo, Ghana and Nigeria (Awono *et al.*, 2002). The species contributes to rural incomes, supplements the local diet and is used in traditional and modern therapies (Schreckenber *et al.*, 2002; Omonhinmin, 2012). The value of the fruit lies in its pulp which is a good source of proteins, fats and carbohydrates that could be used to alleviate malnutrition in children (Ajayi and Adesanwo, 2009; Duru *et al.*, 2012).

Unfortunately, success in breeding for yield superiority of indigenous fruit trees is still constrained by the lack of availability of improved germplasm (Akinnifesi *et al.*, 2007). In fact, managing their populations and improving the quality and regularity of fruit production are perceived as priorities for the economic development of rural populations (Diallo *et al.*, 2008). Thus, controlled-cross-pollination can help to combine some desired characters in the fruits of one tree and increase the high inter-tree variability between selected superior genotypes with high-viability. Wider crosses within a genus often produce favorable proportions of vigorous offspring which can portray

higher germination, seedling growth and survival performances (Batin, 2011).

Moreover, branching in trees is not well understood, but is considered to be a function of apical dominance, the correlative inhibition of axillary buds on shoots of the current year, and apical control, the suppression of existing branch growth imposed by younger more apical shoots (Leakey & Longman, 1986). Branching and crown development in the tropical fruit tree *D. edulis* basically conforms to Rauh's Model (Hallé *et al.*, 1978) and involves both proleptic and sylleptic branching. In the fulfilment of cultivars development for priority tree species, studies on shoot branching which plays a pivotal role in the development of the aboveground plant structure is to be prioritized for future crop diversification programs. Larger size trees are not easily accessible to the grower and it is very risky when climbing to harvest fruits at an appropriate time whereas small-sized trees can be planted closer to each other, more trees per ha implies a further increase in the early harvest. Hence, dwarfed trees are much easier to manage (size, protection measures, harvesting), which significantly reduces production costs per kg of fruit and the risk of accidents (Verheij, 2006). Growth habit is therefore very important in the cropping system of African plum and an influential character in its harvesting. Among the selection criteria for the improvement of this species namely the size and fruit flavour, colour and thickness of the pulp, pulp oil content, the fruiting season, disease resistance and pest, the frequency and regularity of fruiting performance (yield), very little work has been done on the induction of early shoot branching to reduce the size of the tree and thus facilitate the harvesting of fruits and silvicultural management (Kengue, 1990; Kengue *et al.*, 2011). The development of an early selection criterion for early shoot branching would be of particular value in this indigenous species where the techniques used for good quality fruit harvest appear to be strongly related to the plant architecture. Consequently, this paper describes the first application of a decapitation test to this species. Unlike most previous

investigations on timber tree species such as *Triplochiton scleroxylon* K. Schum and *Cedrela odorata* L., (Leakey and Longman, 1986; Lapido et al., 1991) and even perennials such as *Petunia* (Snowden and Napoli, 2003) or procumbent herbs such as *Trifolium repens* L. (Thomas et al., 2003; Thomas and Hay, 2008; Dun et al., 2006), this study was carried out under operational conditions in the nurseries, and it involved screening of large numbers of seedlings rather than clones. To our knowledge, no study has documented *D. edulis* seedlings' growth in response to decapitation. Such approach may help to characterize African plum control-pollinated seedlings in an attempt to select improved raw material for the on-going plant-breeding program. The main objectives were: (i) to assess whether the decapitated test could be successfully applied to *D. edulis*; (ii) to assess whether the responses of this fruit tree to decapitation differed to those observed on timber trees; (iii) to assess the extent of genetic variation in early shoot branching observed using the decapitation test. Thus, the main question addressed was: Do control-pollinated African plum seedlings behavior in the nursery depend on the provenance and the crossing to which they belong?

Materials and methods

Seedlings management in the nursery and the net house at different study sites

For seedlings nurseries activities, the experiment was carried out in the ICRAF's central nursery at Nkolbison (3°51'N Lat., 11°27'E Long.), Cameroon. This site lies at an altitude of 760 m.a.s.l., with a mean annual precipitation of approx.1400 mm and a mean annual temperature of 25, 1°C. From four provenances established in two experimental field trials as living gene banks, control-pollinated seeds were collected from twenty six selected and well-known accessions using a full nested mating design (Makueti et al., 2012a) (Table 1), immediately characterized (Makueti et al., 2012b), labelled and sown directly into black bags (4 l capacity) containing a 2:1 mixture of forest soil and sand. After 28 weeks, the plants were reported into larger buckets (15 l capacity) filled with the same potting mixture at the

ICRAF's experimental net house at Mbalmayo (3°10'N Lat., 11°00'E Long.), for management and evaluation of shoot branching growth as determined by decapitation. This site lies at an altitude of 650 m a.s.l. with a mean annual precipitation of approx. 1802 mm and a mean annual temperature of 24°C. The buckets were treated with fungicide (Ridomil Plus) and insecticide (Cyberdim 220 EC) three days prior to the beginning of the experiment and throughout the seedlings' growth in the net house. The experiment included 13 progenies belonging to 4 provenances. In the net house, the plants (F₁ hybrids) were labelled and arranged at a constant spacing of 35 cm x 35 cm in randomized blocks, with 5 to 6 seedlings per provenance per block arranged in line, and 7 blocks in total, giving a mean total of 42 seedlings per provenance. The buckets were hand-weeded at regular intervals. Seedlings in the net house were grown under shade and watering daily. The sides of the net house were made of shade-cloth allowing 40% ambient light transmission to reduce air temperatures in the net house.

Decapitation test experiments

For dwarfism induction, the seedlings were decapitated on 28 April 2011, 39 weeks after sowing (WAS), by which time they were 46 cm to 70 cm tall. All plants were cut to a constant height of 40 cm as recommended by Leaky and Lapido (1987), by removing the apex or the uppermost node. Cuttings were labelled and taken to the ICRAF's central nursery at Nkolbison for management and evaluation (Makueti et al., in press.). Seedling assessment was carried out at weekly intervals beginning from the 24 day after sowing (DAS). Parameters assessed include: seedling height and shoot length (using meter rule), collar diameter (using veneer caliper), numbers of leaves, numbers of twigs sprouted after decapitation, and numbers of vigorous twigs. These numbers were counted manually. Lengths of lateral shoots (considered in this study as twigs) were measured at two-week intervals over a 6-8 week period. Shoots were considered to be actively growing if they grew by more than 2 mm week⁻¹. There was no lateral shoot present at the time of decapitation. After

decapitation, a solid fertilizer namely 12 g urea (N:P:K; 20:10:10) was added in each bucket.

Statistical analyses

Clustering of accessions into similarity groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. Prior to squared Euclidean distance calculation, the data were standardized by variable to have a mean of zero and a variance of one. Analysis of variance was performed to determine the descriptive statistics such as mean,

standard error, standard deviation and variance for each studied trait. Pearson test was used to assess correlation among variables. A One Way ANOVA test was performed to confirm the accuracy of grouping that produced by cluster analysis. Student-Newman-Keuls Test was used to identify the discriminative traits within and between clusters. We also carried out a linear regression to identify predictors of shoot length's growth. Data were processed under SPSS version 17.0.0 (Aug 23, 2008).

Table 1. List of the 26 studied African plum accessions collected from the two agro-ecologic zones in favour with growth and development in Cameroon. Acc no: Accession number.

Acc. no.	Code in gene bank	Collection sites	Accession sex	Latitude	Longitude	Altitude (m)
AC-01	BUM/DE/26 Seedling 015	Boumnye bel	Female	3 ^o 52'58. 34"N	10 ^o 50'57. 62"E	358
AC-02	BUM/DE/25 Seedling 026	Boumnye bel	Female	3 ^o 52'58. 34"N	10 ^o 50'57. 62"E	358
AC-03	BUM/DE/37 Seedling 111	Boumnye bel	Female	3 ^o 52'58. 34"N	10 ^o 50'57. 62"E	358
AC-04	BUM/DE/25 Seedling 114	Boumnye bel	Female	3 ^o 52'58. 34"N	10 ^o 50'57. 62"E	358
AC-05	BUM/DE/26 Seedling 122	Boumnye bel	Female	3 ^o 52'58. 34"N	10 ^o 50'57. 62"E	358
AC-06	MAK/DE/04 Seedling 078	Makenen e	Female	4 ^o 53'03. 84"N	10 ^o 47'41. 44"E	696
AC-07	MAK/DE/28 Seedling 104	Makenen e	Female	4 ^o 53'03. 84"N	10 ^o 47'41. 44"E	696
AC-08	MAK/DE/01 Seedling 116	Makenen e	Female	4 ^o 53'03. 84"N	10 ^o 47'41. 44"E	696
AC-09	MAK/DE/04 Seedling 144	Makenen e	Female	4 ^o 53'03. 84"N	10 ^o 47'41. 44"E	696
AC-10	KEK/DE/18 Seedling 050	Kekem	Female	5 ^o 09'05. 91"N	10 ^o 01'16. 07"E	715
AC-11	KEK/DE/18 Seedling 070	Kekem	Female	5 ^o 09'05. 91"N	10 ^o 01'16. 07"E	715
AC-12	KEK/DE/07 Seedling 074	Kekem	Female	5 ^o 09'05. 91"N	10 ^o 01'16. 07"E	715
AC-13	KEK/DE/13 Seedling 079	Kekem	Female	5 ^o 09'05. 91"N	10 ^o 01'16. 07"E	715
AC-14	KEK/DE/07 Seedling 142	Kekem	Female	5 ^o 09'05. 91"N	10 ^o 01'16. 07"E	715
AC-15	MAK/DE/35 Seedling1B1	Makenen e	Female	4 ^o 53'03. 84"N	10 ^o 47'41. 44"E	696
AC-16	BUM/DE/14/99	Boumnye	Female	3 ^o 52'58.	10 ^o 50'57.	358

Table 2. Basic statistics for control-pollinated seedlings physiological traits within 39 weeks after sowing (WAS) and 8 weeks after decapitation (WAD); n=108. SH: seedling height; SCD: seedling collar diameter; NLs: number of leaves per seedling; NTAD: number of twigs sprouted after decapitation; NVT: number of vigorous twigs.

Traits	Cluster	N	Minimum	Maximum	Mean	SE	SD	95% Confidence Interval	
								Lower Bound	Upper Bound
SH (cm)	1	16	47.00	66.33	56.95	1.73	6.92	53.26	60.64
	2	9	45.33	66.33	54.59	2.56	7.68	48.68	60.50
	3	11	42.67	67.00	51.39	2.82	9.37	45.09	57.69
SCD (cm)	1	16	3.03	4.27	3.68	0.08	0.35	3.49	3.86
	2	9	2.90	4.20	3.51	0.14	0.42	3.18	3.83
	3	11	2.50	4.03	3.18	0.16	0.55	2.80	3.55
NLs	1	16	4	7	5.35	0.18	0.73	4.96	5.75
	2	9	4	6	5.15	0.17	0.53	4.74	5.56
	3	11	4	5	4.54	0.15	0.50	4.21	4.88
NTAD	1	16	3	7	4.96	0.21	0.85	4.50	5.41
	2	9	3	6	4.52	0.34	0.12	3.73	5.31
	3	11	3	6	4.09	0.28	0.94	3.46	4.72
NVT	1	16	3	5	3.73	0.11	0.44	3.49	3.97
	2	9	3	4	3.41	0.19	.057	2.97	3.85
	3	11	2	4	3.03	0.21	.070	2.56	3.50

Table 3. Means values for control-pollinated seedling characteristics within 39 WAS and 8 WAD (n=108).

Code	Traits	Cluster1	Cluster2	Cluster3	P
T01	Seedling height (cm)	56.95±1.73 ^a	54.59±2.56 ^a	51.39±2.82 ^a	0.216
T02	Seedling collar diameter (cm)	3.68±0.08 ^a	3.51±0.14 ^{ab}	3.18±0.55 ^b	0.023
T03	Number of leave per seedling	5.35±0.18 ^a	5.15±0.53 ^a	4.54±0.50 ^b	0.008
T04	Number of twigs sprouted after decapitation	4.96±0.21 ^a	4.52±0.34 ^a	4.09±0.28 ^a	0.069
T05	Number of vigorous twigs	3.73±0.11 ^a	3.41±0.19 ^{ab}	3.03±0.21 ^b	0.013

Means followed by the same letter within a column are not significantly different at P<0.01 (Student-Newman-Keuls test)

Table 4. Basic statistics for shoot elongation 8 WAD (n=108).

Cluster	Minimum	Maximum	Mean	SE	SD	Variance	N
Cluster 1	10.68	21.87	14.02	0.32	2.22	4.96	48
Cluster 2	8.37	25.80	14.37	0.73	3.81	14.51	27
Cluster 3	9.02	21.25	13.43	0.52	3.01	9.11	33

Results

Description of African plum's control-pollinated seedlings in the nursery

Seedling height (SH) did not showed tree-to-tree variation (P>0.05) in all crosses combined (Table 3), 39 weeks after seeds sowing (WAS). Seedling height mean was 54.66 cm. The highest seedling height was registered from crosses belonging to clusters 1 and 2 respectively (Fig. 1).

Seedling collar diameter (SCD) differed significantly (p<0.05) in all crosses combined between crosses with continuous tree-to-tree variation (Table 3), 39 WAS. Mean seedling collar diameter was 3.48 cm and the biggest collar diameters were registered from crosses belonging to clusters 1 and 2 respectively.

The numbers of leaves per seedling (NLs) differed significantly (p<0.05) between crosses with continuous tree-to-tree variation (Table 3), 39 WAS. Mean number of leaves per seedling was 5.06 and the maximum number of leaves per seedling was

registered from crosses belonging to clusters 1 and 2 respectively.

The number of twigs sprouted after decapitation (NTAD) did not showed tree-to-tree variation ($P > 0.05$) in all crosses combined (Table 3), 8 weeks after decapitation (WAD). Mean number of twigs was 4.58 and the maximum number of twigs sprouted after decapitation was registered from crosses belonging to clusters 1 and 2 respectively.

The numbers of vigorous twigs (NVT) recorded also followed the same trend as obtained for seedling collar diameter and the numbers of leaves ($P < 0.05$) between crosses, 8 weeks after decapitation (WAD). Mean number of vigorous twigs was 3.44. As others studied traits, the maximum number of vigorous twigs was registered from crosses belonging to clusters 1 and 2 respectively.

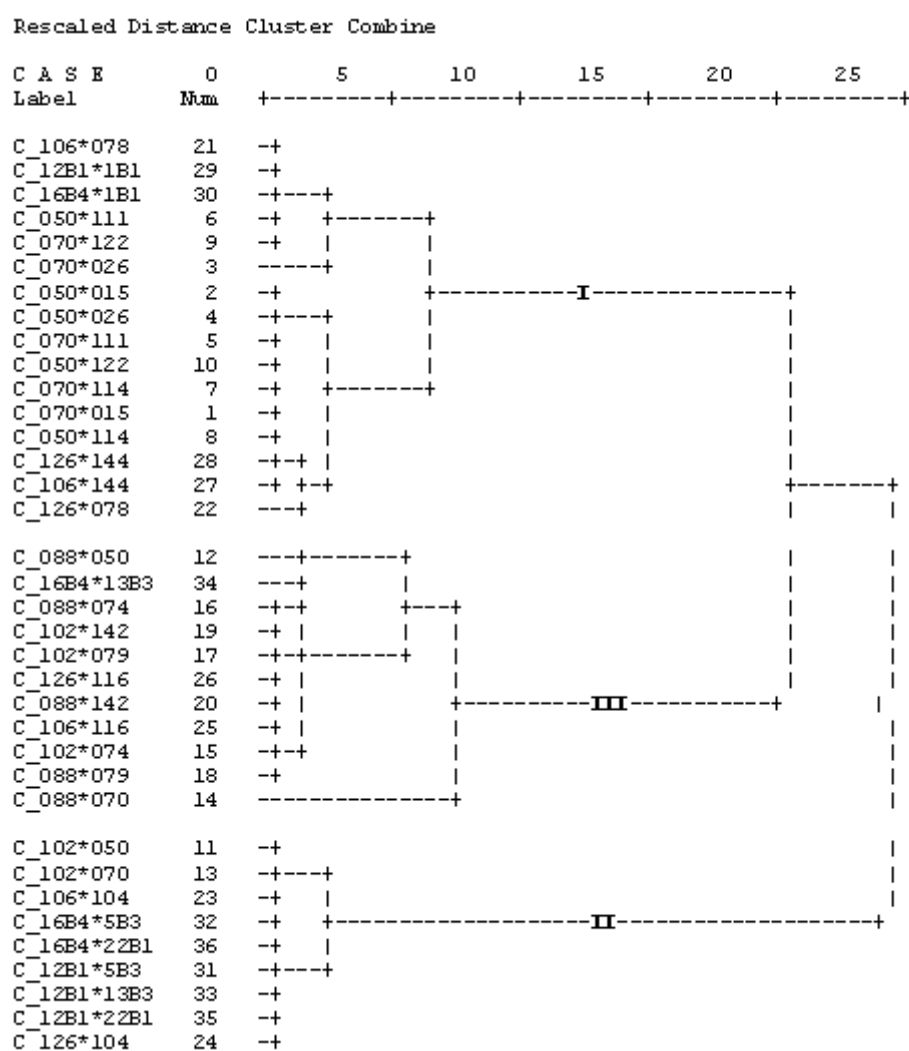


Fig. 1. Ward's dendrogram of 36 crosses on 18 female accessions studied for African plum control-pollinated seedlings characteristics in Cameroon.

Modelling shoot elongation

Regression equations were used to build predictive models for shoot elongation. There were highly significant and strong relationships between seedling high and seedling collar diameter ($R^2 = 0.909$) and

between the numbers of twigs and the numbers of vigorous twigs ($R^2 = 0.899$). However, the numbers of weeks after decapitation (WAD) was a strong predictor of shoot elongation (Fig. 2). The standard

linear regression for African plum decapitated seedling was:

- Shoot length (cm) = -8.808 (0.328) + 0.909 (-2,46) x WAD.....EQ1

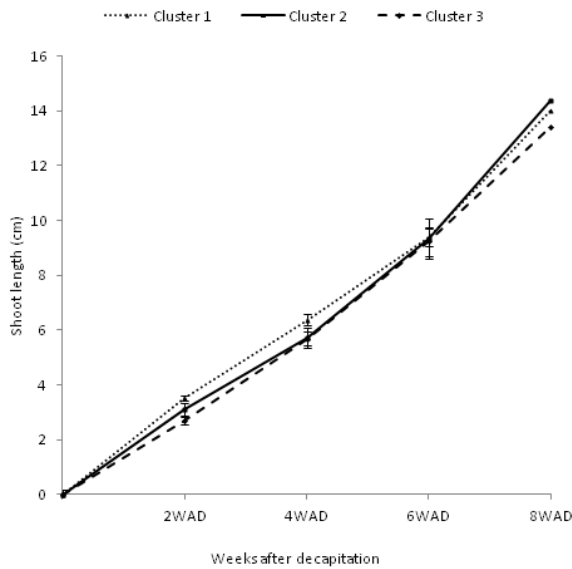


Fig. 2. Shoot length of *Dacryodes edulis* control-pollinated seedlings following decapitation in a net house at Mbalmayo, Cameroon.

Discussion

The variations observed among the seedling growth characteristics such as seedling height, collar diameter, shoot length, numbers of leaves, numbers of twigs sprouted after decapitation and numbers of vigorous twigs can be attributed to the progenies and the crosses to which they belong. This result is in fairly agreement with those reported by Akinngbe and Oni (2007) and Okunlola et al. (2011) respectively on *Prosopis Africana* (Guill., Perrot. And Rich.) and *Parkia biglobosa* (Jack.) Benth. The results of the Analysis of variance (ANOVA) carried out to test the significance of variation at P < 0.01 among the progenies of different provenances using the growth variables revealed that significant variation existed in collar diameter, numbers of leaves and numbers of vigorous twigs. In general, increment in growth variables is an indication of growth, which is a common characteristic of biological organism base on the submission of Fogg (1970). This author asserted that the activities of the various meristematic

cells are correlated; the correlation is such that as the shoot increases in length, it also increases in thickness and the root system extends proportionally. In the present study, it was observed that there was no significant variation in the seedlings’ height. This result is not in line with those reported by Mahoney and Fins (1995), Karaguzel et al. (2004), Upadhaya et al. (2007), Janmohammadi et al. (2008) and Batin (2011). These authors reported that significant variation existed only in seedlings’ height among growth studied variables. According for these authors, seedling height could serve as a trait for identifying genetically superior progenies. Furthermore, strong correlation that exist between height and other growth parameters support that height could serve as a trait for selecting superior progenies. In general, variation in the growth of seedlings may be due to a genetic factor of individual tree and less importantly to soil conditions, soil structures, weed competition and environmental factors.

Previous investigations on some timber trees as *Triplochiton sceroxylon* or *Cedrela odorata* proposed that decapitation test was characterized by apical dominance in young plants which identified two phases of bud activity: the “Sprouting Phase”, in which many buds are released from correlative inhibition, and the “Dominance Phase”, during which (in vertically oriented plants) the uppermost lateral shoots begin to assert dominance and suppress the growth of lower shoots (Phillips, 1969; Leakey and Longman, 1986; Lapido et al., 1991; Newton et al., 1995). Following this conceptual basis for interpretation, it is assumed that clones or seedlings with strong apical dominance release few lateral shoots after decapitation, while those with weak apical dominance sprout more freely. From these previous results, the first assumption indicated the behavior of timber trees’ growth and the second was in line with results from the present study where no growth phases were identified after decapitation. In fact, the decapitation test in *D. edulis* showed synchronal shoot growing in buds released within each cluster. From the relationship between branching habit and harvest index in the studied

species, synchronal shoot growing after decapitation may contribute to dwarfed trees easier to manage. Results for the present study showed that in all crosses combined, there was no significant variation in the numbers of twigs sprouted after decapitation and the number of weeks after decapitation was the only predictor variable for the shoot length among and within clusters. For branching frequency, these results indicate that decapitation test could be used as an early predictive on vigorous offspring which can portray higher germination, seedling growth and survival performances (Diallo et al., 2008). In the fulfillment of cultivars development, this test could be used to screen a large number of seedlings, which could then be selected and vegetatively propagated for establishment in clonal tests. The advantage of screening seedlings in a progeny test design, as in the current investigation, is that genetic values may be estimated for the individual progenies, which may then form the basis of selection.

Conclusion

For a decapitation test in fruit trees, care must be taken to maintain uniformity in the morphological and physiological state of the plants and their growing environment. From this study, plant height seems to be less important as morphological characteristic and the number of weeks after decapitation is the strong predictor for shoot elongation among and within clusters. Moreover, the decapitation test in *D. edulis* showed synchronal shoot growing in buds released within each cluster. From the relationship between branching habit and harvest index in the studied species, synchronal shoot growing after decapitation may contribute to dwarfed trees easier to manage. Further information is clearly required on the relationship between decapitation test results from this study and the eventual field performance of *D. edulis* progenies for the on-going breeding program. Therefore, these decapitated seedlings have been established as seed orchards in a field trial and will be followed up for their field performance management till the first fruiting season.

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