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Full Length Research Paper

In silico prediction and segregation analysis of putative virus defense genes based on SSR markers in sweet potato F1 progenies of cultivars 'New Kawogo' and 'Resisto'

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In sweet potato, an anti-virus defense mechanism termed reversion has been postulated to lead to virus freedom from once infected plants. The objectives of this study were to identify anti-virus defense genes and evaluate their segregation in progenies. Reference genes from different plant species were used to assemble transcript sequences of each sweet potato defense gene *in silico*. Sequences were used for evaluate phylogenetic relationships with similar genes from different plant species, mining respective defense genes and thereafter developing simple sequence repeats (SSRs) for segregation analysis. Eight potential defense genes were identified: RNA dependent RNA polymerases 1, 2, 5, and 6; Argonaute 1, and Dicer-like 1, 2, and 4. Identified genes were differentially related to those of other plants and were observed on different chromosomes. The defense genes contained mono-, di-, tri-, tetra, penta-, and hexa-nucleotide repeat motifs. The SSR markers within progenies were segregated in disomic, co-segregation, nullisomic, monosomic, and trisomic modes. These findings indicate the possibility of deriving and utilizing SSRs using published genomic information. Furthermore, and given that the SSR markers were derived from known genes on defined chromosomes, this work will contribute to future molecular breeding and development of resistance gene analogs in this economically important crop.

Key words: *In silico*, segregation, simple sequence repeats (SSR) markers, sweet potato defense genes, virus.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) production is severely affected by virus diseases that cause yield losses of up to 98% in individual plants (Gibson et al., 1998). Sweet potato is propagated vegetatively using vines as planting material, and farmers use vines from their own crop or traded with other farmers

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(Rachkara et al., 2017) to plant their gardens (Mukasa et al., 2003). The long-standing traditional practice of selecting healthy-looking vine plants as source material, coupled with low levels of symptomatic expression of many single virus infections, have led to the maintenance and proliferation of many viral pathogens (Rachkara et al., 2017). However, the expected high levels of viral prevalence throughout Uganda and consequent reduced yields have not materialized. It has been observed that previously infected field grown plants of East African sweet potato cultivars may become virus free (Adikini et al., 2016); this phenomenon is termed reversion. Similarly, a number of studies have reported that plants previously infected with *Sweet potato feathery mottle virus* became virus free (Green et al., 1988; Abad and Moyer, 1992; Gibb and Podovan, 1993; Gibson et al., 2014).

Gibson and Kreuze (2014) reviewed reversion in East African sweet potato cultivars, and suggested that it is a result of an RNA silencing mechanism triggered in plants by defense genes. The trigger for this plant defense response is the accumulation of viral dsRNA molecules in a replicative form or viral RNA secondary structures. Plant gene products, such as the RNA dependent RNA polymerases (RDRs), are part of the gene silencing machinery that independently synthesizes viral dsRNA in an amplification step for viral small RNA (21 to 24 nts) production. The dsRNAs are processed by Dicer-like (DCL) proteins to small RNAs, which are subsequently incorporated into the RNA-induced silencing complex with an Argonaute (AGO) protein that uses complementary small RNAs to target viral RNA (Baulcombe, 2004; Peragine et al., 2004; Hunter et al., 2016; Leibman et al., 2017). Thus, identification of putative defense genes involved in gene silencing is important in plant breeding for the management of virus diseases.

Defense genes have been studied and identified/ predicted *in silico* in species such as *Nicotiana benthamiana* (Baulcombe, 2004), cucumber (Leibman et al., 2017), potato (Hunter et al., 2016), and cassava (Chellappan et al., 2004); however, the identity and nature of segregation and inheritance of defense genes in sweet potato require investigation. Different methods (webservers) of gene prediction and alignment have been developed. These include, GENOMESCAN (Yeh et al., 2001), AUGUSTUS (Stanke et al., 2004), Open Reading Frame Finder (Wheeler et al., 2003), GENIUS (Puelma et al., 2017), GENEMARK (Lomsadze et al., 2018), GENESCAN (Burge, 1998), Unipro UGENE (Okonechnikoy et al., 2012), CLC workbench (www.qiagenbioinformatics.com/products/clc-main-workbench) among others. Further, plant based bioinformatics tools and databases have been developed, for instance SOL genomics Network (for Solanaceous plants) (Mueller et al., 2005) and Phytozome (for land plants and algae) (Goodstein et al., 2011). In a similar way, *Ipomoea* species tools have recently been developed. These include sweetpotato.plantbiology.msu.edu and sweet potato genome site (public-genomes-ngs.molgen.mpg.de/SweetPotato/). These are useful for understanding the genomics of polyploid sweetpotato. This study thus employed the SOL, Phytozome and *Ipomoea* species bioinformatics tools and GENESCAN, GENOMESCAN, Unipro UGENE and CLC gene prediction platforms, which are majorly open access prediction tools, which would be useful in countries of limited agricultural funding.

One of the methods of studying virus resistance inheritance is through genetic analysis (Mwanga et al., 2002). In this regard, simple sequence repeats (SSRs) are genetic markers that have received particular attention because they are highly informative, codominant, multi-allelic and are experimentally reproducible and transferable among related species (Mason, 2015). SSRs are used for various purposes. These include studies of diversity measured on the basis of genetic distance, evolutionary studies, constructing linkage maps, mapping loci involved in quantitative traits, estimating the degree of kinship between genotypes, marker-assisted selection, defining cultivar DNA fingerprints and estimating gene flow (segregation) in populations (Vieira et al., 2016).

Therefore, this study aimed to identify potential defense genes that may be responsible for reversion against virus infection, and evaluate their segregation patterns using SSR markers.

MATERIALS AND METHODS

Plant

Sweet potato cultivars ‘New Kawogo’ and ‘Resisto’ sourced from virus-free sweet potato collections at the Makerere University Agricultural Research Institute (MUARIK) and Namulonge Crops Resources Research Institute, respectively were used. The white fleshed ‘New Kawogo’ is Ugandan in origin and is virus resistant (Gasura and Mukasa, 2010; Mwanga et al., 2016), while the orange fleshed ‘Resisto’ from the USA is virus susceptible (Mwanga and Ssemakula, 2011). These cultivars were used as parents in a full diallel cross, with reciprocals considered (Griffing, 1956). Resulting seeds were harvested and planted in pots containing sterile potting mix that were then placed in an insect proof screenhouse at MUARIK. Imidacloprid and cypermethrin were applied weekly to control whitefly and aphid vectors of viruses. Each germinated seed was considered a progeny and was grown for 2 months prior to propagation using cuttings that were subsequently established in pots in an insect proof screenhouse at MUARIK.

In silico prediction of defense genes

Defense gene transcript sequences from different plant species were obtained from the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) using Basic Local Alignment Search Tool (BLASTn) (Altschul et al., 1997), and used

as references to derive similar gene sequences for sweet potato. The functions of the reference transcript sequences were verified using the Kyoto Encyclopedia of Genes and Genomes (www.genome.jp/kegg/), the Sol Genomics Network (SOL Genomics.net), and Phytozome (phytozome.net). The reference sequences were BLASTn searched in the Sweet potato genomics resource website (sweetpotato.plantbiology.msu.edu); this process identified homologous sequences within the genomes of the sweet potato relatives *Ipomoea trifida* and *Ipomoea triloba* (Wu et al., 2018). Then, these homologous sequences were used as a template to run a local BLASTn search within a database created in CLC genomic workbench software that was uploaded with a NOTEPAD file of transcript data and chromosomal locations sourced from the Sweet potato genome website (public-genomes-ngs.molgen.mpg.de/SweetPotato/).

Local BLASTn searches were conducted twice during *in silico* evaluation, where the first involved using high stringency parameters with the expectation value set at E^{-10}; transcripts derived at this stringency level were denoted or assigned names depending on number of hits and level of homology. The second search was based on a low level of stringency, with the expectation value set at E^{-6}, and names were assigned as before. This process revealed partial potential sweet potato virus defense gene transcripts and their respective chromosomal locations.

Further, the evolutionary relationship of each defense gene was estimated. This was done using the derived sweet potato virus defense gene transcripts and homologous gene transcripts (of different plant species) sourced from NCBI and sweetpotato.plantbiology.msu.edu. Phylogenetic trees were constructed using maximum likelihood method and following the Jukes and Cantor model (1969) in the CLC workbench. Observations were validated using Unipro UGENE software (Okonechnikov, 2012). Sequences used for rooting the phylogenetic tree were selected randomly.

Partial transcript sequences of sweet potato were also used as templates for mining full DNA sequences from the sweet potato genome website (public-genomes-ngs.molgen.mpg.de/SweetPotato/) using BLATn searching of the sweet potato genome (public-genomes-ngs.molgen.mpg.de/SweetPotato/; Yang et al., 2017). This process product of mining genomic DNA sequences on their respective chromosomes were screened for coding and non-coding regions using the MUSCLE (Edgar, 2004) sequence alignment program on the Unipro UGENE platform (Okonechnikoy et al., 2012). These regions were verified using online tools – GENESCAN, GENOMESCAN and CLC genomic workbench.

Simple sequence repeats mining

DNA sequences within the coding regions were analyzed and screened for simple sequence repeats (SSRs) using WebSat software (wsmartins.net/websat) (Martins et al., 2009). This software was also used to generate SSR-based primers for analysis of segregation of the SSRs in the parental cultivars and their progenies. Outliers (sweet potato cultivars 'Ejumula'and 'Tanzania' and the sweet potato relative *Ipomoea setosa*) were included. Previous work has shown that 'Ejumula' is susceptible to virus infections (Mwanga et al., 2007), while 'Tanzania' is moderately resistant (Gasura and Mukasa, 2010). The virus sensitive *I. setosa* is often used during virus diagnostics in sweet potato (Fuentes, 2010).

Genomic DNA extraction

Genomic DNA of parents, progeny genotypes, and outliers was isolated using a modified version of the CTAB method (Maruthi et al., 2002). DNA quality was established using a NanoDrop-ND-1000 spectrophotometer (Thermo Scientific, Bargal Analytical Instruments, Airport City, Israel), where DNA was diluted to 50 ng and used for downstream analysis. DNA was visualized on 1% agarose gel (VWN International) that was prepared by dissolving it in 0.5% Tris-Borate Acid (TBE) buffer, then warming in a microwave oven, followed by cooling over running tap water. Agarose gels were mixed with ethidium bromide (HyLabs, Rehovot, Israel), cast, and allowed to cool for 30 to 40 min. Then, 5 µl of diluted genomic DNA was mixed with 5 µl of loading dye (prepared using 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol), loaded to the agarose gel, and run in the gel tank for 15 min at 120 V (Clever Scientific, Image Care, Kampala, Uganda).

PCR amplification

Annealing temperature was optimized during amplification of the *in silico* derived primers using a gradient of eight temperatures on a PCR machine (Clever Scientific, Image Care, Kampala, Uganda). The optimal temperature that amplified polymorphic bands was used for subsequent evaluations. The 10 µl PCR master mix contained 3 µl of water, 5 µl of PCR mix (HyLabs Ready Mix [×2], HyLabs, Rehovot, Israel), 0.5 µl of each forward and reverse primer (10 pmol), and 1 µl of DNA (50 ng). The PCR conditions for SSR amplification were an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, extension at 72°C for 30 s, and final extension at 72°C for 10 min. A 3% agarose gel was used for visualization: the gel was warmed in a microwave oven, cooled, and then ethidium bromide was added and mixed. After cooling and setting, the gel was submerged in a gel tank containing 1x TBE buffer. 7 µl of PCR product was mixed with 2 µl of loading dye into the gel that was run at 50 V for 60 min.

Band scoring

Bands were visualized on a gel documentation system (Clever Scientific, Image Care, Kampala, Uganda), where we first analyzed parental cultivars to evaluate and differentiate homozygous from heterozygous bands, according to Guo et al., (2015). A single band was considered to be homozygous at that locus or marker on the gene, while double bands were considered heterozygous. Therefore, primers that revealed double bands in one or both parents were used for progeny segregation analysis. Homozygous SSRs were also used to validate homozygosity in progenies. Bands were scored using binary counts of presence/absence (1/0) criteria.

Segregation evaluation

Segregation was evaluated according to methods used by Zou et al. (2006) and Stift et al., (2008). SSRs that revealed double bands in parental genotypes were considered to be two markers of the same gene; thus, single bands had one marker of that gene. It was assumed that on crossing two markers (that is, A, a), the progeny followed the Mendelian segregation pattern, in a 1:2:1 ratio (1AA:2Aa:1aa). This was considered as disomic inheritance (Zou et al., 2006; Stift et al., 2008) and was revealed as three bands on the gel (Guo et al., 2015). Inheritance mode was classified following Zou et al., (2006), Stift et al., (2008), and Guo et al., (2015), where SSRs that revealed two bands were co-segregating, and zero, one, and four bands were considered as nullisomic, monosomic and tetrasomic inheritance, respectively. Chi-square goodness of fit analysis within XLSTAT (Addinsoft, 2017) was used to test the fit of segregation ratios of SSR markers to the disomic inheritance ratio of 1:2:1 at $P \leq 0.01$.

Table 1. *In silico* prediction of sweet potato defense genes and chromosomal locations (according to genomic data published by Yang et al. (2017).

Gene	Chromosome	Variant identity
IbRDR1	8	*IbRDR1a1*
	8	*IbRDR1a2*
	1	*IbRDR1b2*
	1	*IbRDR1c*
IbRDR2	3	*IbRDR2*
IbRDR5	14	*IbRDR5a*
	11	*IbRDR5b*
IbRDR6	10	*IbRDR6*
IbAGO1	3	*IbAGO1*
IbDCL1	1	*IbDCL1a*
	9	*IbDCL1b*
IbDCL2	12	*IbDCL2a*
	13	*IbDCL2b*
	6	*IbDCL2c*
IbDCL4	8	*IbDCL4*

RESULTS

Defense genes and SSRs

In silico prediction identified eight defense gene families of *I. batatas* (denoted *Ib*) (*IbRDR1*, *IbRDR2*, *IbRDR5*, *IbRDR6*, *IbAGO1*, *IbDCL1*, *IbDCL2*, and *IbDCL4*), located on 10 chromosomes (Table 1). There were six variants of *IbRDR1*; two of these (*IbRDR1a3* and *IbRDR1b1*) were not used (during segregation analysis), because they were highly (98%) homologous, and the other four variants were located on chromosomes 8 and 1 (Table 1). Two variants were found of *IbRDR5* located on chromosomes 14 and 11; two variants of *IbDCL1* located on chromosomes 1 and 9; and, three variants of *IbDCL2* located on chromosome number 12, 13, and 6. There were no variants of the remaining genes (Table 1).

Abundance of SSRs

Mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats were detected within the various coding regions of the defense genes. Pentanucleotide repeats were the most abundant (52.04%), while the least abundant were trinucleotide and tetranucleotide repeats (both were 4.09%) (Table 2). The highest proportion of repeats was observed in *IbRDR1a2* (44.67%), while the lowest was in *IbRDR6* (0.44%); and all forms of repeat were observed in *IbRDR1a2* (Table 2).

Phylogenetic relationships of sweet potato defense genes to similar genes in other plant species

The phylogenetic relationship revealed that some putative sweet potato (*I. batatas* - *Ib*) defense genes or their variants had recently evolved and are either closely related to *I. trifida* or *I. triloba*; yet distantly related to those of other plant species.

The relationship of six species of *IbRDR1* varied. The *IbRDR1a1*, *IbRDR1a2, IbRDR1a3* evolved earlier than other *IbRDR1s*, though diverged from *I. triloba RDR1* variant 1. The *IbRDR1b2* and variant *IbRDR1a4* appeared to have recently evolved. All the *IbRDR1s* are related to variants of either *I. trifida*, *I. triloba* or *Ipomoea nil* RDR1 (Appendix Figure 1). The *RDR1* variants of other plant species like *Cucurbita* species, *Nicotiana* species, *Hevea brasillensis*, *Manihot esculenta* and *Oleo europaea* (Appendix Figure 1) are distantly related to *I. batatas RDR1* and its variants. Also, according to the phylogram, *I. batatas RDR2* recently evolved from *I. trifida* and *I. triloba*; though share a common ancestor

Table 2. Analysis of polymorphisms (repeats) in coding regions of the defense genes.

Gene	Variant	Number of repeats						Cumulative repeats	Proportion (%)
		Mono	Di	Tri	Tetra	Penta	Hexa		
IbRDR1	*IbRDR1a1*	0	1	0	0	2	3	6	2.46
	IbRDR1a2	20	11	8	8	37	25	109	44.67
	IbRDR1b2	0	0	0	0	3	1	4	1.64
	IbRDR1c	0	1	0	0	5	0	6	2.46
IbRDR2	*IbRDR2*	0	0	0	0	4	0	4	1.64
IbRDR5	*IbRDR5a*	0	1	0	0	1	0	2	0.82
	IbRDR5b	1	1	0	1	12	3	18	7.38
IbRDR6	*IbRDR6*	0	0	0	0	1	0	1	0.44
IbAGO1	*IbAGO1*	0	1	0	0	3	1	5	2.05
IbDCL1	*IbDCL1a*	2	3	1	0	11	3	20	8.19
	IbDCL1b	2	0	1	1	19	8	31	12.70
IbDCL2	*IbDCL2a*	1	0	0	0	10	2	13	5.33
	IbDCL2b	0	0	0	0	2	1	3	1.23
	IbDCL2c	0	0	0	0	1	1	2	10.82
IbDCL4	*IbDCL4*	1	1	0	0	16	2	20	8.19
Cumulative repeats	-	27	20	10	10	127	50	-	-
Abundance (%)	-	11.1	8.19	4.09	4.09	52.04	20.49	-	-

Mono: Mononucleotide repeats; Di: dinucleotide repeats; Tri: trinucleotide repeats; Tetra: tetranu-cleotide repeats; Penta: pentanucleotide repeats; Hexa: hexanucleotide repeats.

(*I. nil RDR2*). All variants of *Ipomoea RDR2* diverged from the *RDR2* of *Nicotiana*, *Solanum* and *Capsicum* species. Also, *RDR2* of root crop *M. esculenta* and fruit crop *Vitis vinifera* evolved earlier than *I. batatas RDR2*. These *RDR2* also diverged extensively from *I. batatas RDR2* (Appendix Figure 2).

When *RDR5* of different plants was estimated, it was observed that *RDR5* of all *Ipomoea* spp. evolved earlier than the *RDR5* of other plant species. The *IbRDR5b* evolved earlier than *IbRDR5a*. The *IbRDR5b* clustered with *I. triloba RDR5* variants yet *IbRDR5a* clustered with those of *I. nil* and *I. trifida* (Appendix Figure 3). On the other hand, the *RDR6* of *Nicotiana* and *Solanum* spp. evolved much earlier than that of *Ipomoea* spp. Regarding the respective *Ipomoea* spp., *I. batatas RDR6* evolved earlier than *RDR6* of *I. trifida*, *I. triloba* and *I. nil* (Appendix Figure 4).

The *I. batatas AGO1* and *I. trifida AGO1* are closely related and share *I. nil AGO1* as a phylogenetic ancestor. Further, whereas the *AGO1* of all *Ipomoea* spp. is related to the *AGO1* of *Nicotana* and *Solanum* spp., they diverged earlier from those of fruit trees like *V. vinifera* and *Citrus sinensis* among others (Appendix Figure 5). Additionally, with the exception of *DCL1* from *Solanum* spp. and *Nicotiana tabacum*, the *DCL1* of *Ipomoea* spp. has recently evolved. In particular, *I. batatas DCL1b* and *IbDCL1a* evolved earlier than *DCL1* of *I. trifida* or *I. triloba*; though highly related. The *DCL1* of other species sampled (for instance *Theobroma cacao*, *Hevea brasiliensis*, *M. esulenta* among others) evolved much earlier than *I. batatas DCL1* (Appendix Figure 6).

The phylogram showed that *I. batatas DCL2b* and *IbDCL2c* diverged from *I. nil DCL2* and its variants. The *IbDCL2b* and *IbDCL2c* evolved earlier than *DCL2* from *I. triloba* and *I. trifida.* The *IbDCL2a* has recently evolved though related to *I. triloba* and *I. trifida*. The *DCL2* of plants like *Capsicum annum* and *Solanum lycopersicum* evolved earlier than that of *Ipomoea* spp. (Appendix Figure 7). The *I. batatas DCL4* is closely related to *I. triloba* and have recently evolved. The *DCL4* of other plant species evolved much earlier than *I. batatas DCL4.* The *DCL4* of *Solanum* and *Nicotiana* spp. evolved much earlier than those of *Ipomoea* spp. (Appendix Figure 8).

Segregation analysis of defense genes using SSRs

From a total of 222 SSR generated primers, 63 SSR

Table 3. Heterozygous defense gene SSR variants and their respective markers.

Gene	Variant and chromosome (chr) location	Primer name	Repeat	Primer sequence (5′- 3′) Forward and Reverse	Identifier
ibRDR1	*IbRDR1a1* (Chr 8)	*IbRDR1a1_3*	(TTTATT)2	GGCCACATGGTAAATGAAGTAT GTGTTTTGAGGGCTGTTAATGT	Marker A
	IbRDR1a2 (Chr 8)	*IbRDR1a2_17*	(TA)11	AAGCTGTAAGCACGGAGTAAAA AGAAGAAGAAGAAGAAGGAGGG	Marker B
	IbRDR1a2 (Chr 8)	*IbRDR1a2_74*	(A)13	GCATTAGCGCATTACTGGTT AACACGATAAAGAAGATGAGGC	Marker C
IbDCL1	*IbDCL1a* (Chr 1)	*IbDCL1a_7*	(TTCAA)2	GGGTTGAAACACCTAGTAATGC AGCTGTGTGGAGGGTTAGTTTA	Marker D
	IbDCL1a (Chr 1)	*IbDCL1a_15*	(TA)10	GGGGTCATTTCTGTATGTGATT GTCCCTGCTTCAAAGGTAAGAT	Marker E
	IbDCL1b (Chr 9)	*IbDCL1b_23*	(AGTAGC)2	TTAACTGAAACCCTAGCCTCAC GCATCAAGTCAACTCAACTCAA	Marker F
	IbDCL1b (Chr 9)	*IbDCL1b_24*	(ATA)6	TTAACTGAAACCCTAGCCTCAC GCATCAAGTCAACTCAACTCAA	Marker G
IbDCL2	*IbDCL2b* (Chr 13)	*IbDCL2b_3*	(AGTAAA)2	GCAAGAATCGAATTTAGTGCTC TTCCCGAAATGTCTACTGCTAT	Marker H
	IbDCL2c (Chr 6)	*IbDCL2c_2*	(AGTAAA)2	GCAAGAATCGAATTTAGTGCTC TTCCCGAAATGTCTACTGCTAT	Marker I

primer sets were used in downstream analysis, from which nine showed heterozygous bands when evaluated in the parent cultivars, and were assumed to represent markers (Table 3).

From the nine heterozygous SSRs on the different chromosomes, we identified 449 alleles in the 50 progenies, among which 51.44% segregated monosomically, 37.27% were co-segregated, and 9.3% fitted the expected disomic inheritance model; trisomic and nullisomic segregation was low (1.55 and 0.44%, respectively) (Table 4). There was deviation ($P \leq 0.01$) from the disomic inheritance model for segregation of all markers (Table 4).

Inheritance of the defense gene SSRs varied within the progenies, as indicated by the different models of segregation for the markers (Table 4). Inheritance models of markers were found as follows: B, D, and C were disomic, co-segregation, monosomic, and trisomic; E was disomic, co-segregation, nullisomic, and monosomic; F, G, and I were disomic, co-segregating, and monosomic; A was disomic and co-segregating; and H was co-segregating and monosomic. Co-segregation inheritance dominated for markers A, D, and C, while monosomic inheritance dominated for markers B, I, and E. Marker A had the highest proportion of co-segregating progenies (96%), while marker E had the lowest proportion of nullisomic progenies (2%) (Table 4).

DISCUSSION

Using *in silico* predictions from the sweet potato genome (Yang et al., 2017; Wu et al., 2018), we identified sweet potato putative defense genes, their variants and their microsatellites (SSR markers) and evaluated their segregation patterns. This is the first study of SSRs from specific chromosome locations, gene coding or involved in virus RNA silencing, and their segregation as potential virus defense gene markers in sweet potato progenies. Eight putative defense genes were derived using high and low stringency cut-off values; low stringency prediction has previously been used to derive resistance genes in sugarcane (Wanderley-Nogueira et al., 2007) and to identify defense gene variants (Table 1) in *I. trifida*

Table 4. Test for progeny segregation of putative virus defense gene SSRs in a population of 50 seed progeny crosses between 'New Kawogo' and 'Resisto'.

Marker	Model 1:2:1	Progeny deviation from model/alternate models				Test for deviation from the disomic model (Chi-square)
	%Disomic (2n+1)	%Co-segregation (2n)	%Nullisomic (2n-2)	%Monosomic (2n-1)	%Tetrasomic (2n+2)	
A	4 (2)	(48) 96	-	-	-	16*
B	16 (8)	(13) 26	-	(27) 54	(2) 4	41*
C	28 (14)	(30) 60	-	(3) 6	(3) 6	15*
D	24 (12)	(30) 60	-	(6) 12	(2) 4	18.667*
E	2 (1)	(4) 8	(2) 4	(43) 86	-	67.667*
F	4 (2)	(14) 28	-	(34) 68	-	50*
G	2 (1)	(14) 28	-	(35) 70	-	51.33*
H	-	(7) 14	-	(43) 86	-	59.669*
I	4 (2)	(8) 16	-	(41) 82	-	57.333*
Total	9.3 (42)	(168) 37.25	(2) 0.44	(232) 51.44	(7) 1.55	-

Data in parentheses are number of progenies. *Segregation of each marker deviated from the fitted model (Chi-square values for 50 progenies) at P ≤ 0.01.

and *I. triloba* (Wu et al., 2018), *I. nil* (Morgulis et al., 2008), and potato (Hunter et al., 2016). The defense genes of sweet potato were phylogenetically related to defense genes in other plants (Appendix Figures 1 to 8). Specifically, there was close relationship within *Ipomoea* spp. This is confirmed by a related report that was made by Feng et al., (2018) about the evolutionary relationship between *I. batatas* (sweet potato) and wild relatives *I. trifida* and *I. triloba*.

In the present study, the detection of microsatellites (SSR markers) within the DNA coding regions of the defense genes indicates an improvement in understanding of defense genes and virus resistance compared with previous knowledge (Mwanga et al., 2002; Yada et al., 2017). It is important to note that the sweet potato SSRs currently known (Parado, 2010; Wang et al., 2011) are randomly located within the genome and tend to be difficult to develop or study without the use of sophisticated equipment (Schafleitner et al., 2010; Wang et al. 2011); however, the method used in the present is inexpensive and targeted to specific genes and chromosomes. It was found that pentanucleotide repeats were the most abundant (52.04%), followed by hexanucleotide repeats (20.49%) (Table 2). In contrast, hexanucleotide repeats are most frequent (46.38%) in arum lily (*Zantedeschia aethiopica*), followed by mono-repeats (31.86%) (Radhika et al., 2011), trinucleotide repeat motifs dominate in citrus and jatropha (Wen et al., 2010), and di-nucleotide repeats dominate in potato (Tang et al., 2009). This study is the first to report the presence of all major forms of repeat motif (mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide) within *IbRDR1*, demonstrating a considerable increase in the number of available genetic markers for sweet potato.

Segregation of putative sweet potato defense genes was analyzed in progenies, and found it tended to be disomic, co-segregating, nullisomic, monosomic, and tetrasomic (Table 4), confirmed by deviations from the expected Mendelian segregation ratio of 1:2:1 (P ≤ 0.01). This deviation may be complex, because no clear pattern of segregation of defense genes was found in sweetpotato. In a study of progeny from different parental crosses, Mwanga et al. (2002) reported that resistance genes segregate in both Mendelian and non-Mendelian patterns, and we suggest this may have occurred in the present study (Table 4). The present results is in contrast with those reported by Rukarwa et al., (2013) for segregation of the *cry7Aa1* gene for weevil resistance in sweetpotato, which segregated in a Mendelian pattern, as would usually be expected for a transgene. When each marker was considered, some progenies inherited genes disomically and fitted well to the Mendelian segregation model (Table 4), indicating almost perfect crossing, whereas other progenies had chromosome doubling (1.55% tetrasomic inheritance) or reduction (0.44% nullisomic inheritance). Interestingly, varied forms of segregation were found within a marker in different progenies (Table 4), indicating that marker segregation in sweet potato may be highly variable among progenies. It is also possible that the allelic composition of a particular defense gene varies among progenies, where it could be an underlying factor in the variability of reversion potential in different sweet potato genotypes (Wasswa et al., 2011; Gibson et al., 2014) and in the variability of disease and pest resistance in potato (Yermishin et al., 2016). Variable patterns of segregation in progenies may also be attributed to segregation distortion of different genes and chromosomes during crossing, as has been reported for barley (Liu et al., 2011) and coffee (Ky et al., 2000), possibly because the large sweet potato chromosome number (2n=6x=90) may be subject to segregation

distortion and a high level of cross incompatibility (Knox and Ellis, 2002; Yamagishi et al., 2010).

The breeding of provitamin A-rich orange-fleshed sweet potato with virus resistance is a priority in East Africa (Low et al., 2017). There are, therefore, immediate opportunities for use of this resistance marker gene technique in crop breeding, as demonstrated here, that includes crossing parents with important characteristics (virus resistant, white flesh 'New Kawogo' and virus susceptible, orange flesh 'Resisto'). The approaches used here may be easily applied to SSR markers for the provitamin A synthetic pathway (Wu et al., 2018) for further development of sweet potato cultivars.

Conclusion

Identification of putative virus resistance genes in the sweet potato genome and development of SSR markers using bioinformatics tools is potentially more efficient than using traditional methods. The SSRs detected in this study may be used in molecular breeding and development of resistance gene analogs, and gene clustering studies of this culturally and economically important crop. This detection of important defense genes in polyploid sweet potato suggests this may be equally possible for other complex genomes, like those of potato and peanut.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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REFERENCES

Abad JA, Moyer JW (1992). Detection and distribution of sweet potato feathery mottle virus in sweet potato by in vitro transcribed RNA probes (riboprobes), membrane immunobinding assay and direct blotting. Phytopathology 82:300-305.

Addinsoft (2017). Data analysis and statistical solutions for Microsoft Excel. Addinsoft, Paris, France.

Adikini S, Mukasa SB, Mwanga ROM, Gibson RW (2016). Effects of sweet potato feathery mottle virus and sweet potato chlorotic stunt virus on yield of sweet potato in Uganda. Journal of Phytopathology 164:242-254.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25:3389-3402.

Baulcombe D (2004). RNA silencing in plants. Nature 431:356-363.

Burge CB (1998). Modeling dependencies in pre-Mrna splicing signals. In Salzberg S, Searls D, Kasif S eds. Computational methods in Molecular Biology. Elsevier Sceience, Amsterdam pp.127-163.

Chellappan P, Vanitharani R, Fauquet CM (2004). Short interfering RNA accumulation correlates with host recovery in DNA virus-infected hosts, and gene silencing targets specific viral sequences. Journal of Virology 78:7465-7477.

Edgar RC (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acid Research 32:1792-1797.

Feng JY, Li M, Zhao S, Zhang C, Yang ST, Qiao S, Tan WF, Qu HJ, Wang DY, Pu ZG (2018). Analysis of evolution and genetic diversity of sweet potato and its related different polyploidy wild species I. trifida using RADseq. BioMed Central Plant Biology 18:181.

Fuentes S (2010). Sweet potato virus indexing procedure - OP23. International Potato Center (CIP).

Jukes TH, Cantor CR (1969). Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, Academic Press, New York pp. 21-132.

Gasura E, Mukasa SB (2010). Prevalence and implications of sweet potato recovery from sweet potato virus disease in Uganda. African Crop Science Journal 18:195-205.

Gibb KS, Podovan AC (1993). Detection of sweet potato feathery mottle virus in sweetpotato grown in northern Australia using an efficient and simple assay. International Journal of Pest Management 39:223-228.

Gibson RW, Kreuze JF (2014). Degeneration in sweet potato due to viruses, virus-cleaned planting material and reversion: a review. Plant Pathology 64:1-15.

Gibson RW, Mpembe J, Alicia T, Carey EE, Mwanga ROM, Seal SE, Vetten HJ (1998). Symptoms, etiology and serological analysis of sweet potato virus diseases in Uganda. Plant Pathology 47:95-102.

Gibson RW, Wasswa P, Tufan HA (2014). The ability of cultivars of sweet potato in East Africa to 'revert' from sweet potato feathery mottle virus infection. Virus Research 186:130-134.

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012). Phytozome: A comparative platform for green plant genomics. Nucleic Acid Research 40:1178-1186.

Green SK, Kuo YJ, Lee DR (1988). Uneven distribution of two potyviruses (sweet potato feathery mottle virus and sweet potato latent virus) in sweet potato plants and its implication on indexing of meristem-derived plants. Tropical Pest Management 34:298-302.

Griffing B (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Sciences 9:463-493.

Guo Y, Wu Y, Anderson JA, Moss JQ, Zhu L (2015). Disomic inheritance and segregation distortion of SSR markers in two populations of *Cynodon dactylon* (L.) Pers. var. dactylon. PLoS ONE 10:e0136332.

Hunter LJR, Brockington SF, Murphy AM, Pate AE, Gruden K, MacFarlane SA, Palukaitis P, Carr JP (2016). RNA-dependent RNA polymerase 1 in potato (*Solanum tuberosum*) and its relationship to other plant RNA dependent RNA polymerases. Scientific Reports 6:23082.

Knox MR, Ellis THN (2002). Excess heterozygosity contributes to genetic map expansion in pea recombinant inbred populations. Genetics 162:861-873.

Ky CL, Barre P, Lorieux M, Trouslot P, Akaffou S, Louarn J, Charrier A, Hamon S, Noirot M (2000). Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.). Theoretical and Applied Genetics 101:669-676.

Leibman D, Kravchik M, Wolf D, Haviv S, Weissberg M, Ophir R, Paris HS, Palukaitis P, Ding SW, Gaba V, Gal-on A (2017). Differential expression of cucumber RNA-dependent RNA polymerase 1 genes during antiviral defence and resistance. Molecular Plant Pathology 19:300-312.

Liu X, You J, Guo L, Liu X, He Y, Yuan J, Liu G (2011). Genetic analysis of segregation distortion of SSR markers in F2 population of barley. Journal of Agricultural Science 3:172-177.

Lomsadze A, Gemaye K, Tang S, Borodovsky M (2018). Modeling leaderless transcription and atypical genes results in more accurate gene prediction in prokaryotes. Genome Research 28:1079-1089.

Low JW, Mwanga ROM, Andrade M, Carey E, Ball AM (2017). Tackling vitamin A deficiency with biofortified sweet potato in sub-Saharan Africa. Global Food Security 14:23-30.
Martins WS, Lucas DCS, de Souza Neves KF, Bertioli DJ (2009). WebSat - A web software for microsatellite marker development. Bioinformation 3:282-283.
Maruthi MN, Colvin J, Seal S, Gibson G, Cooper J (2002). Co-adaptation between cassava mosaic geminiviruses and their local vector populations. Virus Research 86:71-85.
Mason AS (2015). SSR Genotyping. In: Batley J, editor. Plant Genotyping. Springer; New York, NY: pp. 77–89.
Morgulis AR, Coulouris G, Raytselis Y, Madden TL, Agarwala R, Schafffer AA (2008). Database indexing for production MegaBLAST searches. Bioinformatics 24:1757-1764.
Mukasa SB, Rubaihayo PR, Valkonen JPT (2003). Incidence of viruses and virus like diseases of sweet potato in Uganda. Plant Disease 87:329-335.
Mwanga ROM, Kriegner A, Cervantes-Flores JC, Zhang DP, Moyer JW, Yencho GC (2002). Resistance to sweet potato chlorotic stunt virus and sweet potato feathery mottle virus is mediated by two separate recessive genes in sweet potato. Journal of the American Society for Horticultural Science 127:798-806.
Mwanga ROM, Kyalo G, Ssemakula GN, Niringiye C, Yada B, Otema MA (2016). NASPOT 12 O" and "NASPOT 13 O" sweetpotato. HortScience 51:291-295.
Mwanga ROM, Odongo B, Niringiye C, Alajo A (2007). Release of two orange-fleshed sweet potato cultivars, 'SPK004' ('Kakamega') and 'Ejumula', in Uganda. HortScience 42:1728-1730.
Mwanga ROM, Ssemakula G (2011). Orange-fleshed sweetpotatoes for food, health and wealth in Uganda. International Journal of Agricultural Sustainability 9:42-49.
Mueller LA, Solow TH, Taylor N, Skwareck B, Buels R, Binns J, Lin C, Wright MH, Ahrens R, Wang Y, Herbst EV, Keyder ER, Menda N, Zamir D, Tanksley SD (2005). The SOL Genomics Network: a comparative resource for Solanaceae biology and beyond. Plant Physiology 138:1310-1317.
Okonechnikoy K, Golosova O, Fursov M (2012). The UGENE team. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28:1166-1167.
Parado KH (2010). SSR for sweetpotato. www. Research.cip.cgiar.org. Accessed 2/11/2018.
Peragine A, Yoshikawa M, Wu G, Albrecht HL, Poethig RS (2004). SGS3 and SGS2/ SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. Genes and Development 18:2368-2379.
Puelma T, Araus V, Canales J, Vidal E, Cabello J, Soto A, Gutierrez R (2017). GENIUS: Web server to predict local gene networks and key genes for biological functions. Bioinformatics 33:760-761.
Rachkara P, Phillips DP, Kalule SW, Gibson RW (2017). Innovative and beneficial informal sweet potato seed private enterprise in northern Uganda. Food Security 9:595-610.
Radhika V, Aswath C, Lakshman Reddy DC, Bhardwaj SA (2011). *In silico* microsatellite development in arum lily (*Zantedeschia aethiopica*). Journal of Horticultural Science 6:37-40.
Rukarwa RJ, Mukasa SB, Sefasi A, Ssemakula G, Mwanga ROM, Ghislain M (2013). Segregation analysis of cry7Aa1 gene in F1 progenies of transgenic and non-transgenic sweet potato crosses. Journal of Plant Breeding and Crop Science 5:209-213.
Schafleitner R, Tincopa LR, Palomino O, Rossel G, Robles RF, Alagon R, Rivera C, Quispe C, Rojas L, Pacheco JA, Solis J, Cerna D, Kim JY, Hou J, Simon R (2010). A sweet potato gene index established by denovo assembly of pyrosequencing and Sanger sequences and mining for gene-based microsatellite markers. BMC Genome 11:604.
Stanke M, Steinkamp R, Waack S, Morgenstern B (2004). AUGUSTUS: A web server for gene finding in eukaryotes. Nucleic Acids Research 32 (Web Server issue) W309-12.
Stift M, Berenos C, Kuperus P, van Tienderen PH (2008). Segregation models for disomic, tetrasomic and intermediate inheritance in tetraploids: A general procedure applied to *Rorippa* (Yellow Cress) microsatellite data. Genetics 179:2113-2123.
Tang S, Okashah RA, Cordonnier-Pratt M, Prat LH, Johnson VE, Taylor CA, Arnold ML, Knapp SJ (2009). EST and EST-SSR marker resources for Iris. BMC Plant Biology 9:72.
Vieira MLC, Santini L, Diniz AL, Munhoz CF (2016). Microsatellite markers: what they mean and why they are so useful. Genetics and Molecular Biology 39:312-28.
Wanderley-Nogueira AC, Soares-Cavalcanti NM, Morais DAL, Belarmino LC, Barbosa-Silva A, Benko-Iseppon AM (2007). Abundance and diversity of resistance genes in the sugarcane transcriptome revealed by *in silico* analysis. Genetics and Molecular Research 6:866-889.
Wang Z, Li J, Luo Z, Huang L, Chen X, Fang B, Li Y, Chen J, Zhang X (2011). Characterization and development of EST-derived SSR markers in cultivated sweet potato (*Ipomoea batatas*). BMC Plant Biology 11:139.
Wasswa P, Otto B, Maruthi MN, Mukasa SB, Monger W, Gibson RW (2011). First identification of a sweet potato begomovirus (sweepovirus) in Uganda: characterization, detection and distribution. Plant Pathology 60:1030-1039.
Wheeler DL, Church DM, Federhen S, Lash LE, Madden TL, Pontius JU, Schuler GD, Schriml LM, Sequeira E, Tatusova TA, Wagner L (2003). Database resources of the National Center for Biotechnology. Nucleic Acid Research 31:28-33.
Wen M, Wang H, Xia Z, Zou M, Lu C, Wang W (2010). Development of EST-SSR and genomic-SSR markers to assess genetic diversity in *Jatropha curcas* L. BMC Research Notes 3:42.
Wu S, Lau KH, Cao Q, Hamilton JP, Sun H, Zhou C, Eserman L, Gemenet D, Olukolu B, Wang H, Crisovan E, Godden GT, Jiao C, Wang X, Kitavi M, Manrique-Carpintero N, Vaillancourt B, Wiegert-Rininger K, Yang X, Bao K, Zheng Y, Schaff J, Kreuze J, Gruneberg W, Khan A, Ghislain M, Ma D, Jiang J, Mwanga ROM, Leebens-Mack J, Coin LJM, Yencho GC, Buell CR, Fei Z (2018). Genome sequences of two diploid wild relatives of cultivated sweet potato reveal targets for genetic improvement. Nature Communications 9:4580.
Yada B, Alajo A, Ssemakula GN, Mwanga ROM, Brown-Guedira G, Yencho GC (2017). Selection of Simple Sequence Repeat Markers Associated with Inheritance of Sweet potato Virus Disease Resistance in Sweetpotato. Crop Science 57:1421-1430.
Yamagishi M, Takeuchi Y, Tanaka I, Kono I, Murai K, Yano M (2010). Segregation distortion in F2 and doubled haploid populations of temperate japonica rice. Journal of Genetics 89:237-241.
Yang J, Moeinzadeh MH, Kuhl H, Helmuth J, Xiao P, Haas S, Liu G, Zheng J, Sun Z, Fan W, Deng G (2017). Haplotype-resolved sweet potato genome traces back its hexaploidization history. Nature Plants 3:696-703.
Yeh RF, Lim LP, Burge CB (2001). Computational inference of homologous gene structures in the human genome. Genome Research 11:803-816.
Yermishin AP, Svitoch OV, Voronkova EV, Gukasian ON, Luksha VI (2016). Determination of the composition and the allelic state of disease and pest resistance genes in potato parental lines using DNA markers. Russian Journal of Genetics 52:498-506.
Zou JJ, Singh RJ, Lee J, Xu SJ, Hymowitz T (2006). SSR markers exhibit trisomic segregation distortion in soybean. Crop Breeding and Genetics 46:1456-1461.

APPENDIX

Phylograms showing evolutionary relationships of defense genes of sweet potato to other plant species.

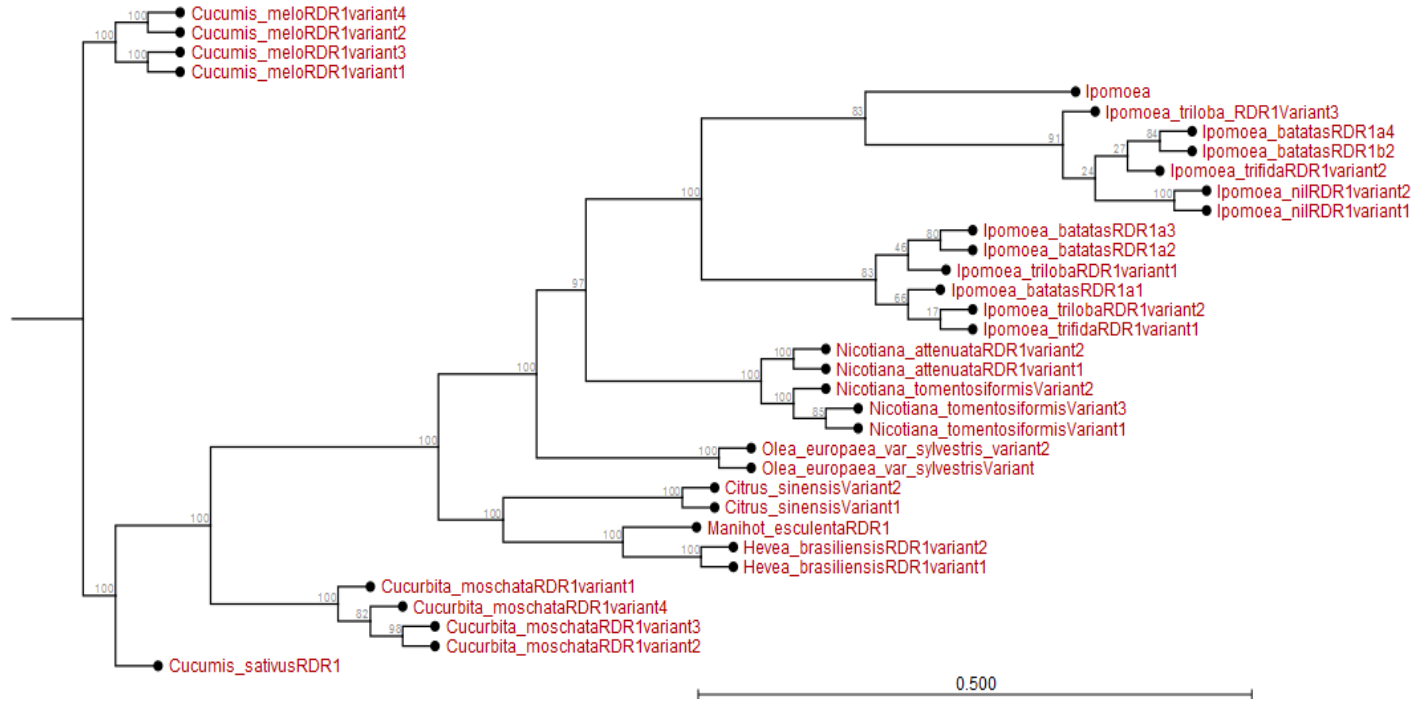

Figure 1. Phylogram showing evolutionary relationship of *Ipomoea batatas RDR1* and *RDR1* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

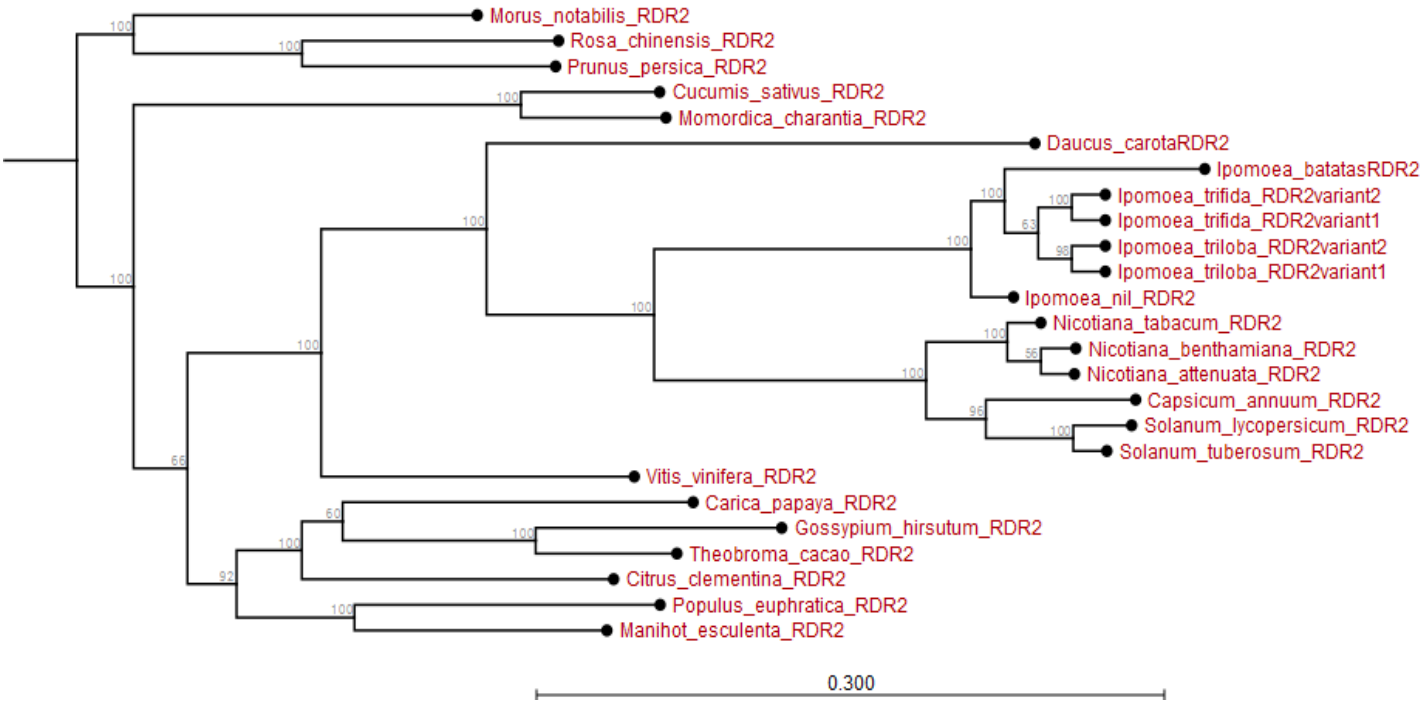

Figure 2. Phylogram showing evolutionary relationship of *Ipomoea batatas RDR2* and *RDR2* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

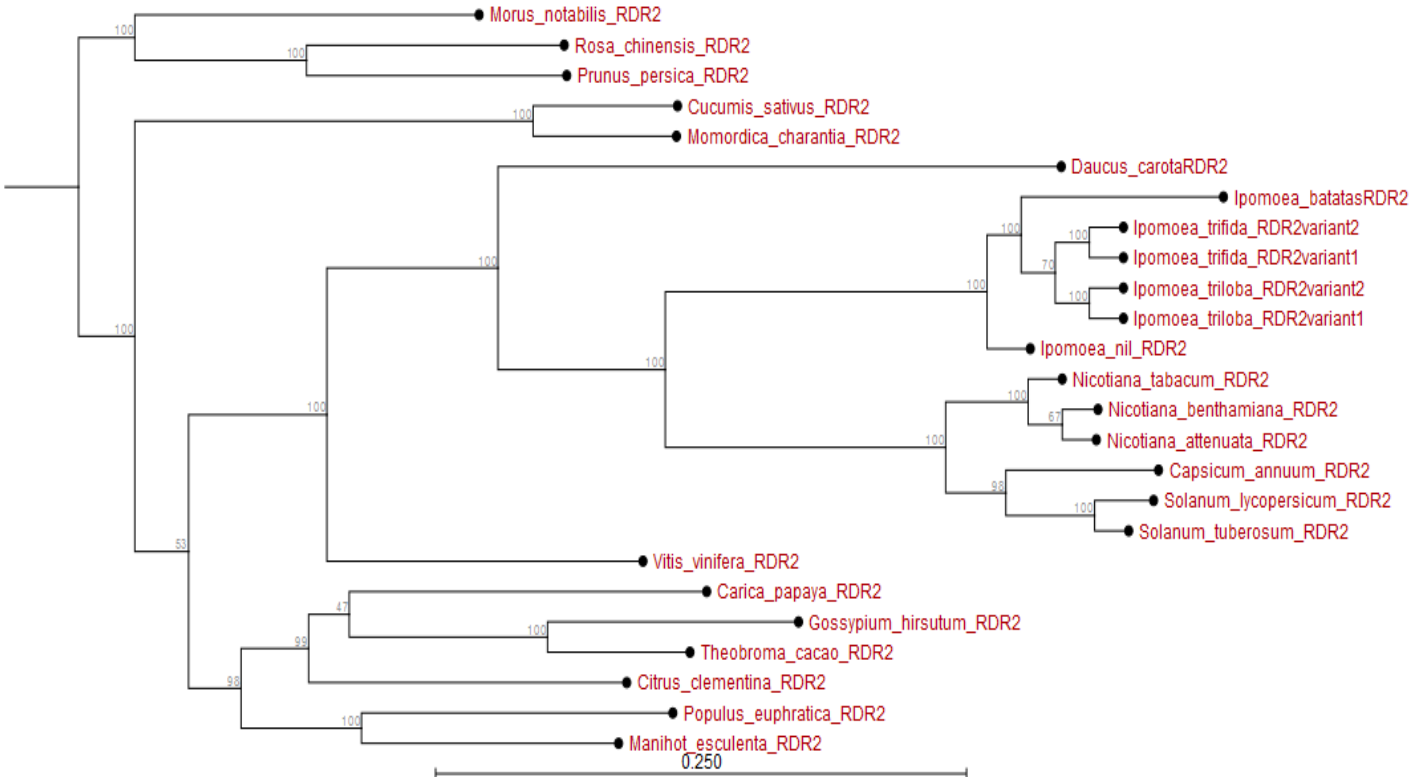

Figure 3. Phylogram showing evolutionary relationship of *Ipomoea batatas RDR5* and *RDR5* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

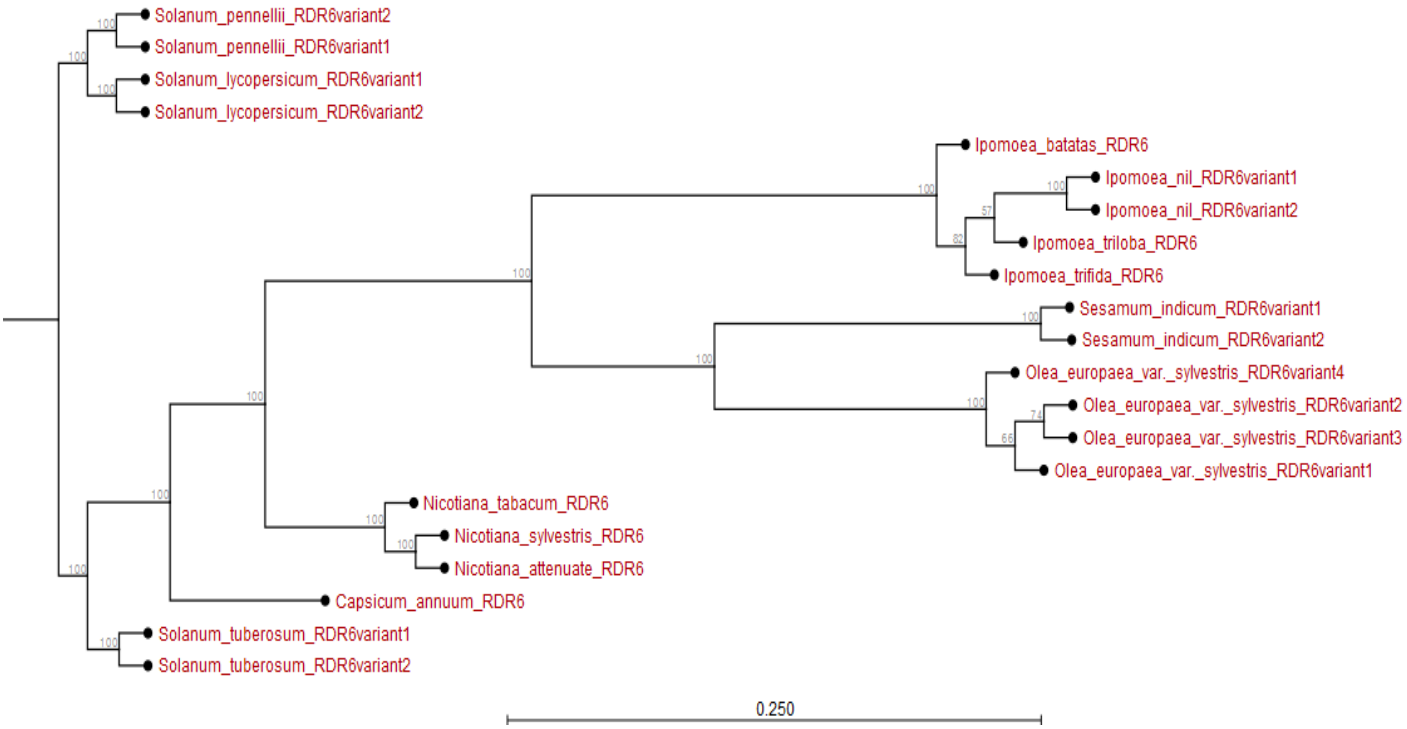

Figure 4. Phylogram showing evolutionary relationship of *Ipomoea batatas RDR6* and *RDR6* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

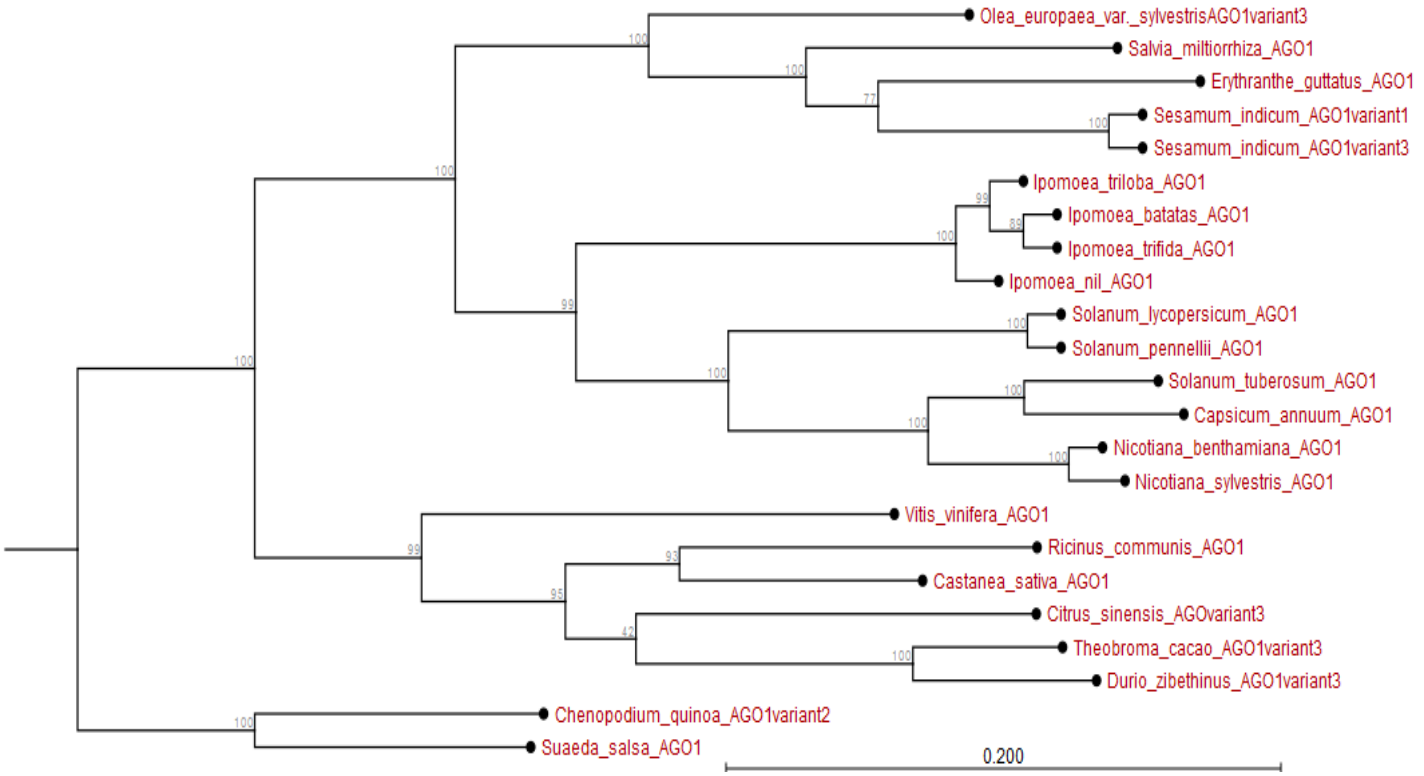

Figure 5. Phylogram showing evolutionary relationship of *Ipomoea batatas AGO1* and *AGO1* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

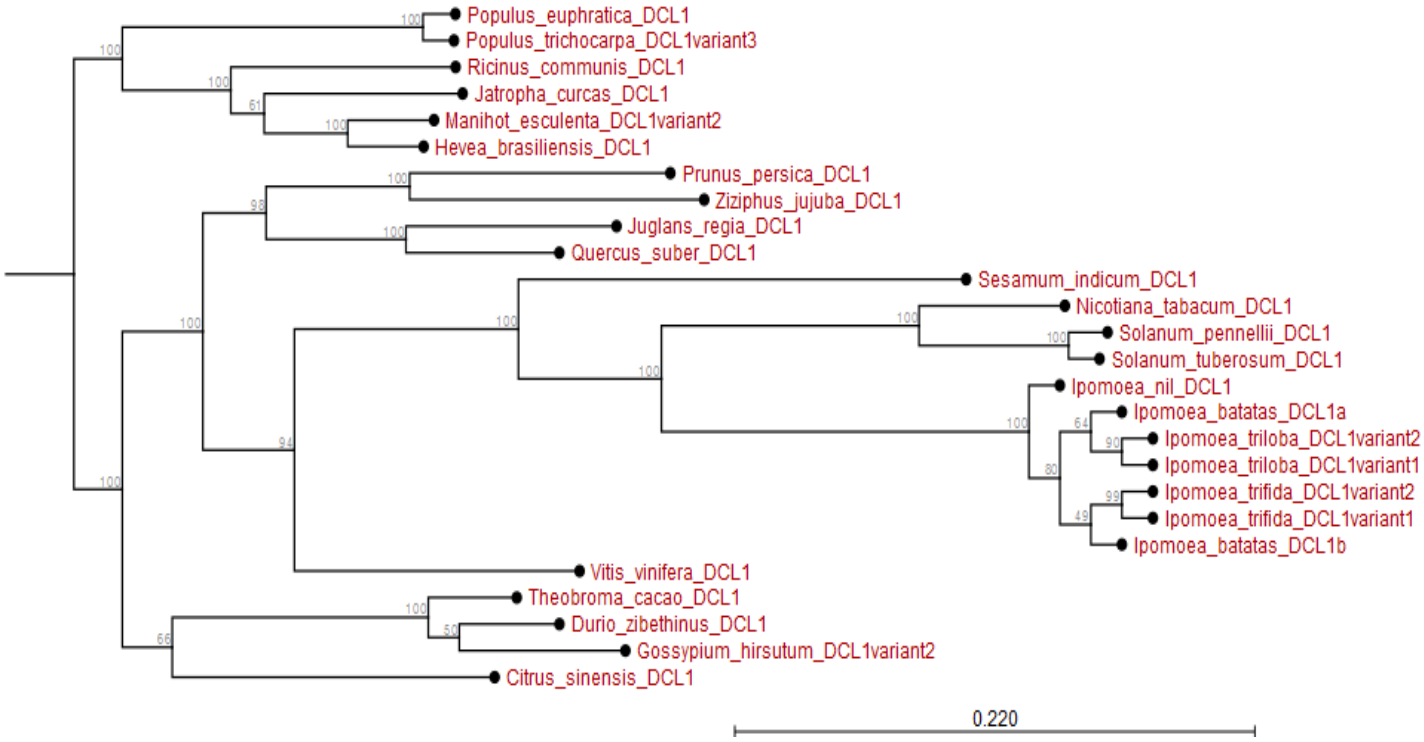

Figure 6. Phylogram showing evolutionary relationship of *Ipomoea batatas DCL1* and *DCL1* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

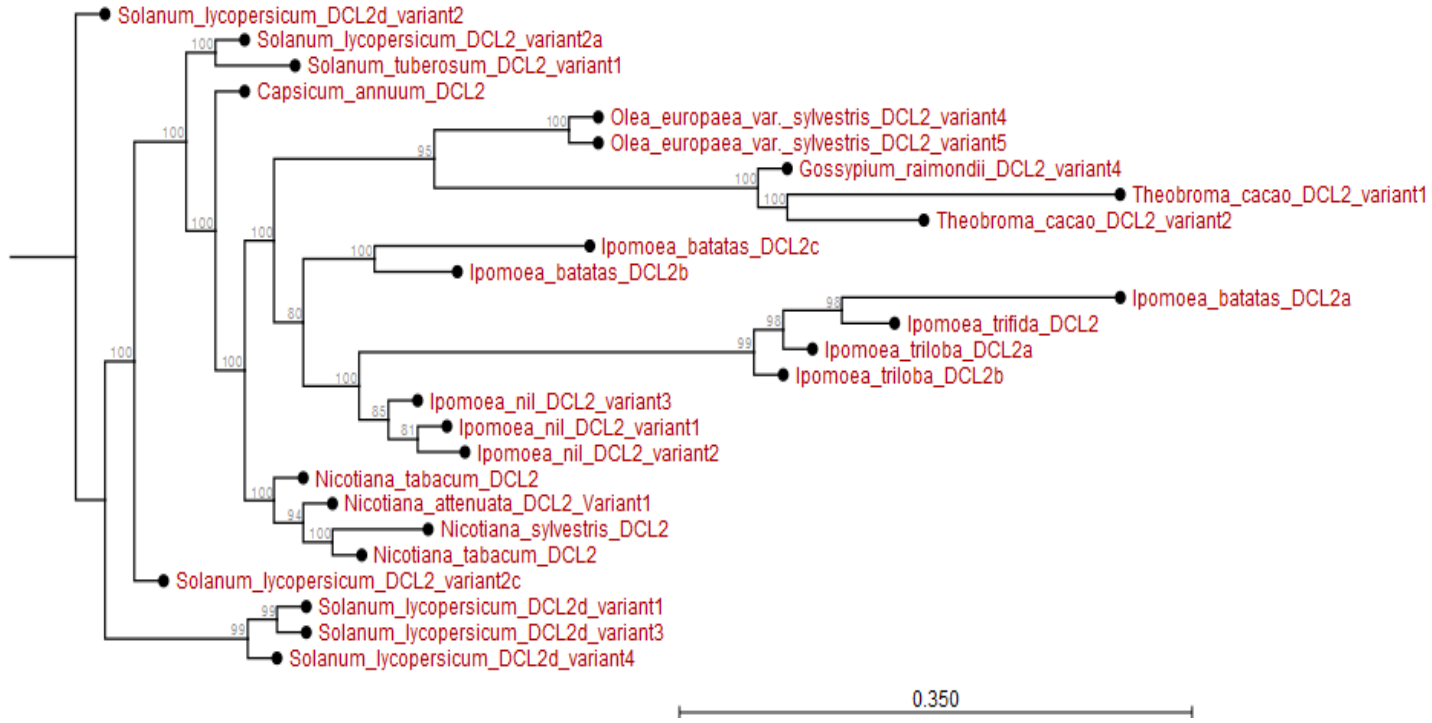

Figure 7. Phylogram showing evolutionary relationship of *Ipomoea batatas DCL2* and *DCL2* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

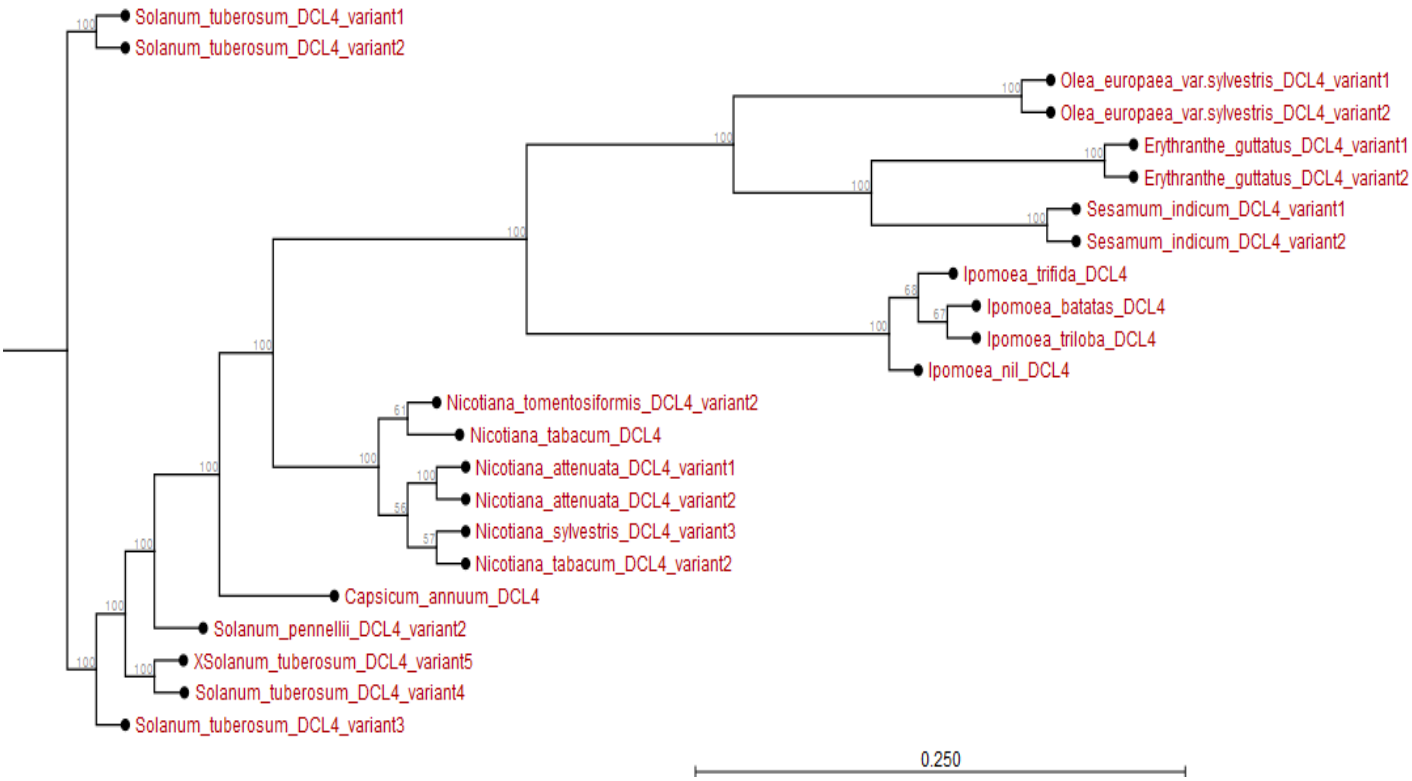

Figure 8. Phylogram showing evolutionary relationship of Ipomoea batatas *DCL2* and *DCL2* from plant species sampled from NCBI and sweetpotato.plantbiology.msu.edu.

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Early selection in *Eucalyptus camaldulensis* Dehnh. progenies in Savanna, Brazil

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Genetic parameters were assessed and correlated to evaluate the feasibility of early selection for diameter at breast height (DBH) and total height (TH) in a half-sib progenies test of *Eucalyptus camaldulensis*. The test is established in the Santo Antônio do Leverger municipality, Mato Grosso State, Brazil, with 132 progenies, five blocks and three plants per plot at 3 x 2 m spacing. REML/BLUP procedure was applied at three and six years of age of trees for estimation. Analysis of deviance revealed no significance for progeny effects for variable TH in both age groups. Genetic parameters were of low magnitude and gains were almost zero. However, for variable DBH, significance in the analysis of deviance was recorded. The accuracies were of high magnitude and there was low heritability variation for the assessed ages. Gains with direct selection for DBH were 38.03% (DBH 3) and 34.86% (DBH 6), the genetic correlation between the two ages was high and positive (0.958) and the indirect selection gain was 33.65%, indicating a situation favourable to early selection. The results will subsidize the first breeding program of *E. camaldulensis* in the Mato Grosso State.

Key words: Forest tree improvement, half-sib progenies, indirect selection.

INTRODUCTION

Eucalyptus (*Eucalyptus* spp.) is the most planted forest tree species in Brazil. Rapid growth, wood production to different uses, adaptability to different soil and climatic conditions, associated with the intense improvement and optimization of silvicultural practices programs, had facilitated the expansion of the culture and its cultivation in the country (Gonçalves et al., 2013). Currently, *Eucalyptus* species are planted on 5.7 million hectares in Brazil (IBÁ, 2017). *Eucalyptus grandis*, *Eucalyptus urophylla* and their hybrids are predominantly cultivated (Pires et al., 2011) but other species like *Eucalyptus camaldulensis* Dehnh., also has potential for productive plantations.

E. camaldulensis is a species that presents a wood that

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is employed in civil construction due to its high density, and also in the manufacture of paper, cellulose, wood-based panels, woodwork and carpentry, transmission poles, firewood, coal and wooden sleepers (Santos et al., 2008; Butcher et al., 2009). This species also has the capacity of growing in critical geographic zones such as in moderately poor soils, and is resistant to long periods of drought and concentrated rain, similar characteristics found in the savanna biome and also in many regions of Mato Grosso State (Azevedo et al., 2015) – a state located in the Center-West region of Brazil.

Though the Eucalyptus is the forest species most planted in Mato Grosso State (IMEA, 2013), there are no eucalyptus improvement program especially for this State. The reason may be due to the distance from commercial centres and the absence of great forest businesses that work with the genus. Thus, the State's plantations are established with genetic materials not adapted to its soil and climatic conditions. Consequently, it is necessary to seek alternatives to enable the selection of adequate genetic material in a short period.

In early selection model, several selection criteria can be analyzed and this technique is important for breeding programs because of the advantages it presents. Associated with repeatability, this model makes it possible to select with greater certainty the best material to obtain genetic gains in the first years of plant development (Wu, 1998, 1999; Apiolaza, 2009).

In genetic improvement programs of forest species such as the *Eucalyptus*, the time factor is an important variable, due to its long productive cycle (Moraes et al., 2014b). Hence, the early selection presents itself as the main alternative for reducing the time of the improvement cycle, once it is carried out before rotation age with the aim of minimizing the generation interval, and consequently, increasing genetic gain without losing productivity (Marques Junior, 1995; Fujimoto et al., 2006).

The efficiency of early selection is allied to the high correlation of characteristics assessed at juvenile age and rotation age (Ferreira, 2005). Studies assessing the feasibility of early selection model for the *Eucalyptus* genus demonstrate the possibility of this practice (Chaves et al., 2004; Massaro et al., 2010; Lima et al., 2011; Beltrame et al., 2012; Apiolaza et al., 2013; Denis and Bouvet, 2013; Moraes et al., 2014a; Pavan et al., 2014; Pinto et al., 2014; Costa et al., 2015; Tambarussi et al., 2017).

In this context, the present work aimed to assess the possibility of carrying out early selection of potential clones in the first progeny test of *E. camaldulensis* located in the savanna of Mato Grosso State, Brazil.

MATERIALS AND METHODS

Progeny and research area

Progeny test was conducted in the experimental field of the Federal Institute of Education, Science and Technology of Mato Grosso (IFMT, Campus São Vicente) in the municipality of Santo Antônio do Leverger, Mato Grosso State, Brazil. The area is located in the biome savanna, at latitude 15°49'21"S and longitude 55°25'06"W, with 750 m of altitude and 2000 mm of annual average precipitation. According to the Köppen-Geiger classification, the climate of the region is of the *Aw type*, with average temperature of 20°C.

For establishment of the experiment, seeds of *E. camaldulensis* were collected from open-pollinated trees at Faculty of Engineering, UNESP Campus Ilha Solteira (provenance Katherine River, Queensland, Australia) in partnership with Embrapa Forests and Federal University of Mato Grosso (UFMT).

The half-sib progenies test was established under randomized block design with 132 treatments (progenies), five repetitions and three plants per plot in single lines at 3 x 2 m spacing. At three and six years old, the circumferences at breast height (in centimeters) and total height (TH; in meters) of all trees of the experiment were measured with the aid of a tape measure and the Blume-Leiss hypsometer, respectively. For data analysis, circumference values were converted into diameter at breast height (DBH), and the values divided by π.

Statistical analysis

In both ages, the estimates of the components of genetic parameters variances were obtained by the restricted maximum likelihood method and best linear unbiased prediction (REML/BLUP) on the basis of unbalanced data and employing the Selegen-REML/BLUP genetic-statistical software developed by Resende (2002). The model used was *model 1*, which is suitable for half-sibling progeny tests under randomized block design, with several plants per plot, only one site and a single population. The referred model is given by "$y = Xb + Za + Wc + e$", where y = data vector; b = vector of block effects (fixed); a = vector of additive genetic effects (random); c = vector of plot effects (random); e = vector of random errors; X, Z and W = incidence matrices for b, a and c, respectively.

The effects of genotypes and plots were tested via analysis of deviance through the *chi-square test* at 1 and 5% of probability, comparing deviance values with and without the respective effect.

In order to aid the assessment of the feasibility of early selection, the genetic correlation between the two measurements was also estimated by Selegen-REML/BLUP.

Estimates of genetic gains with direct and indirect (early) selection were obtained by the employment of the following expressions, cited by Cruz et al. (2004):

(i) $GS_{i(x)}\,(\%) = \frac{GSi}{\bar{x}} \times 100$, where $GS_{i(x)} = DS_{(x)} \times h^2_{a(x)}$

(ii) $GS_{i(x,y)}\,(\%) = \frac{DS(x,y)}{\bar{x}} \times 100$, where $GS_{i(x,y)} = DS_{(x,y)} \times h^2_{a(x,y)}$

where, $GS_{i(x)}$ (%) = genetic gain estimated at age "x" by the selection practiced directly on character "i" at the same age; $GS_{i(x,y)}$ (%) = genetic gain estimated at age "x" by the selection practiced directly on character "i" at age "y", with:$h^2_{a(x)}$ = individual heritability at age "x"; $h^2_{a(x,y)}$ = individual heritability at age "x" based on the selection of individuals at age "y"; $DS_{(x)}$ = direct selection – selection differential at age "x";$DS_{(x,y)}$ = indirect selection – selection differential at age "x" based on the selection of individuals at age "y".

We obtained the percentage of coincidence between the best individuals in both ages by ranking the 75 best individuals at three years of age, having observed the frequency of these 75 individuals at the age of six.

Table 1. Analysis of deviance (ANADEV) for diameter at breast height (DBH) and total height (TH) in progenies of *Eucalyptus camaldulensis* at three and six years of age.

Effect / age	DBH 3		DBH 6		TH 3		TH 6	
	Dev.	LRT(X^2)	Dev.	LRT(X^2)	Dev.	LRT(X^2)	Dev.	LRT(X^2)
Progeny	3861.6	16.33**	4046.9	18.15**	2924.03	$1.43^{n.s.}$	3469.08	$0.08^{n.s.}$
Complete model	3845.2		4028.8		2922.6		3469.0	
Overall average	10.63 cm		12.40 cm		9.33 m		10.47 m	

Dev. = deviance; LRT = likelihood ratio test; fixed X^2 = 3.84 and 6.63, for significance levels of 5 and 1%, respectively; **p < 0.05, *p < 0.01, n.s. = not significant.

Table 2. Genetic parameters for DBH (diameter at breast height in centimeters) at 3 and 6 years of age from progeny test of *E. camaldulensis*.

Parameter	DBH 3	DBH 6
h^2_a	0.37 ± 0.11	0.36 ± 0.10
h^2_{mp}	0.53	0.6
Ac_{prog}	0.73	0.7
CV_{gi}%	22.38	21.2
CV_e%	23.77	21.2
CV_r%	0.94	1.00

h^2_a: Block narrow-sense individual heritability, that is, additive effects; h^2_{mp}: Heritability of the progeny mean; Ac_{prog}: Accuracy of progeny selection; CV_{gi}%: Coefficient of variation of progenies; CV_e%: Coefficient of experimental variation; CV_r%: Coefficient of relative variation.

RESULTS AND DISCUSSION

For the DBH variable, significance was observed for progeny effects at 5% of probability (Table 1), and thus it is possible to infer that there is genetic variability to practice selection on the basis of DBH both at three and six years of age. As for height, there was no significant observation for progeny effects in both ages by the analysis of deviance, which indicates low genetic variation for this variable and lower gain perspective. Because of this, the other analyses (gain estimates and genetic parameters) will not be presented due to their near-zero values.

Several authors have shown the existence of genetic variability in characters associated with growth in genetic tests with eucalyptus species of three years old, both in early selection of clones (Massaro et al., 2010; Beltrame et al., 2012; Moraes et al., 2014b) and early selection in progeny tests (Henriques et al., 2017). The manifestation of this variability in younger ages favors the early selection process allowing the identification of superior genotypes prior to rotation age.

However, besides the fact that there is genetic variability for the selection in early ages to occur, it is important that the best genotypes be kept in the first positions of the ranking, both at rotation age and at the age that one intends to perform early selection. In the present study, the coincidence between the best genotypes in the two ages (three and six years old) was 86%, showing that there was little change between the two ages. Additionally, the genetic correlation for DBH in the two assessed ages was 95.8%, which means that the selection of individuals' superior in DBH at three years old shall be close or similar to the age of six.

The experimental variation coefficient (CV_e%) demonstrates that good experimental precision and its estimates in the present work were around 20% (Table 2), values superior to those found by other authors in eucalyptus progeny tests, as in Freitas et al., (2009) at three years old and Moraes et al. (2015) at 36 months old in different localities. However, the genetic variation presented a magnitude close to environmental variation, once the relative variation coefficient (CV_r) that expresses the relation between the experimental variation (CV_e%) and the progeny variation coefficient (CV_{gi}%) was close or equal to 1 (3 and 6 years old, respectively) (Table 2), which evidenced a favourable situation for selection (Vencovsky and Barriga, 1992; Cruz et al., 2004).

The observed accuracies (Table 2) were of high magnitude for both ages (values superior to 0.7) (Resende and Duarte, 2007). This result indicates that the selection based on this character is safe by virtue of the high relation between the predicted and real value.

The block narrow-sense individual heritabilities (h^2_a) estimated in the experiment were superior to those reported by Henriques et al. (2017) in a progeny test of

Table 3. Direct and indirect gains and selection mean for the 75 best individuals (potential clones) in progeny test of *E. camaldulensis*.

X	Y	MP (cm)	MS (cm)	SG	ISG
DBH 3	DBH 3	10.63	14.67	38.03%	-
	DBH 6	12.40	16.57	-	33.65%
DBH 6	DBH 3	-	-	-	-
	DBH 6	12.40	16.72	34.86%	-

X: Age at which selection was practiced; Y: Ages of response to selection; MP: Original population mean in centimeters; MS: Individuals means selected in order to practice direct and indirect selection in centimeters; SG: Selection gain; ISG: Indirect selection gain.

E. urophylla, with the 0.15 value at three years of age. Progenies average heritability estimates (h^2_{mp}) were consistent with values found by Rocha et al*.* (2006) with progenies at 58 months of age and Moraes et al*.* (2014a) who assessed at the ages of 24, 36, 48 and 60 months in clonal tests of *Eucalyptus* spp., where the values were superior to 0.7.

We verified that, in general, heritability values for the two assessed ages were of medium magnitude (Resende and Duarte, 2007); also, they presented low variation with age (Table 2), which indicates that there was no strong change of genotypic variance in relation to the environmental in the two assessed ages. Some authors report that heritability increases with age, which leads to infer that there is a greater influence of environment on juvenile characteristics (Borges et al*.*, 1980; Kalil Filho et al*.*, 1982) or that, insofar as trees become adults, the genotype exerts greater influence on the expression of phenotype (Borges et al*.*, 1980), a fact that was verified by Henriques et al*.* (2017), who assessed seven years old *E. urophylla* progenies and Beltrame et al*.* (2012) with *Eucalyptus* spp. hybrid clones at three and seven years old. Especially in this study, the maintaining of heritability values in different ages is another factor that favors selection, so that the estimated gains in the two ages have similar values since an early period and the same selection intensity is maintained.

Direct genetic gains with the selection of potential clones were similar in the two ages. This situation was also reported by Pinto et al*.* (2014) with *E. urophylla* clones at the ages of three and seven (Table 3). Genetic gain with indirect selection by three years old was 33.65%. Thus, we observe that, when practicing indirect selection and selecting the DBH character by three years of age with the aim to obtain results in the DBH character at six years, the values are similar to those of the direct selection gain, which confirms the possibility of the early selection of progenies at age of three.

Previous experiments with Eucalyptus have shown this relation between selection gains, which provide good estimates for early selection. Massaro et al*.* (2010), with the prediction of genetic gains by 25 months, 50 months and 72 months in *Eucalyptus* spp. clones, indicated that genetic gains maintained close values between the ages on the basis of the DBH variable. Araújo (2015) also verified the potentiality of early selection in two clonal tests of *E. grandis* x *E. urophylla* hybrids and obtained estimates that prove that the selection of individuals at two years old results in genetic gain in adult age (72 months old).

Conclusion

Existence of genetic variability in the selection for DBH at three and six years of age, associated with the closeness of the estimated indirect gain values with selection at three years old, and the high correlation between genotypic values for DBH in the two assessed ages, indicate that the process of selection at an age inferior to that of rotation proved to be viable, not compromising gains and identifying the best genetic materials early. These results will subsidize the first breeding program of *E. camaldulensis* in the Mato Grosso State, Brazil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Apiolaza LA (2009). Very early selection for solid wood quality: screening for early winners. Annals of Forest Science 66:601-611.https://doi.org/10.1051/forest/2009047

Apiolaza L, Chauhan S, Hayes M, Nakada R, Sharma M, Walker J (2013). Selection and breeding for wood quality: A new approach. New Zealand Journal of Forestry 58(1):32-37.

Araújo MJD, Dias DC, Scarpinati EA, Paula RC (2015). Number of replicates, of plants per plot and of evaluations for eucalyptus clonal tests. Pesquisa Agropecuária Brasileira 50(10):923-931.http://dx.doi.org/10.1590/S0100-204X2015001000008

Azevedo LPA, Costa RB, Martinez, DT, Tsukamoto Filho AA, Brondani GE, Baretta MC, Ajala WV (2015). Genetic selection in *Eucalyptus camaldulensis* progenies in savanna area of Mato Grosso State, Brazil. Ciência Rural 45(11):2001-2006. http://dx.doi.org/10.1590/0103-8478cr20131557

Beltrame R, Bisognin DA, Mattos BD, Cargnelutti Filho A, Haselein CR, Gatto DA, Santos GA (2012). Silvicultural performance and early selection of clones from eucalyptus hybrids. Pesquisa Agropecuária Brasileira 47(6):791-796. http://dx.doi.org/10.1590/S0100-204X2012000600009

Borges RCG, Brune A, Silva JC, Regazzi AJ (1980). Estimativa de parâmetros genéticos em *Eucalyptus grandis* W. Hill ex-Maiden. Revista Árvore, Viçosa 4(2):134-145.

Butcher PA, McDonald MW, Bell JC (2009). Congruence between environmental parameters, morphology and genetic structure in Australia's most widely distributed eucalypt, *Eucalyptus camaldulensis*. Tree Genetics and Genomes 5:189-210. https://doi.org/10.1007/s11295-008-0169-6

Chaves JH, Reis GG, Reis MGF, Neves JCL, Pezzopane JEM, Polli HQ (2004). Early selection of eucalypt clones to be planted in environments with varying soil water availability: water relations of plants in small size plastic tubes. Revista Árvore 28(3):333-341. http://dx.doi.org/10.1590/S0100-67622004000300003

Costa RB, Martinez DT, Silva JC, Almeida BC (2015). Variability and genetic gains in *Eucalyptus camaldulensis* progenies using different selection methods. Revista de Ciências Agrárias 58(1):69-74. https://doi.org/10.4322/rca.1668

Cruz CD, Regazzi AJ, Carneiro PCS (2004). Modelos biométricos aplicados ao melhoramento genético. 3 ed. Editora Viçosa: UFV, 480p.

Denis M, Bouvet JM (2013). Efficiency of genomic selection with models including dominance effect in the context of *Eucalyptus* breeding. Tree Genetics and Genomes 9(1):37-51. https://doi.org/10.1007/s11295-012-0528-1

Ferreira AR (2005). Genetic analysis and selection in *Pinus taeda* L. progeny tests from diallel designs. Ph.D. dissertation, Sector of Agrarian Sciences, Federal University of Paraná, Curitiba/PR, Brazil. Available in: http://www.floresta.ufpr.br/pos-graduacao/defesas/pdf_dr/2005/t176_0188-D.pdf

Freitas RD, Vasconcelos ED, Cruz CD, Rosado AM, Rocha RB, Takami LK (2009). Prediction of genetic gains in open pollinated *Eucalyptus urograndis* cultivated in different environments and submitted to different selection procedures. Revista Árvore 33(2):25-263. http://dx.doi.org/10.1590/S0100-67622009000200007

Fujimoto T, Akutsu H, Kita K, Uchiyama K, Kuromaru M, Oda K (2006). Age trends of genetic parameters of spiral grain in hybrid larch F1 and implications for efficiency of early selection. Journal of Wood Science 52(2):101-106.https://doi.org/10.1007/s10086-005-0735-6

Gonçalves JLM, Alvares CA, Higa AR, Silva LD, Alfenas AC, Stahl J, Ferraz SFB, Lima WP, Brancalion PHS, Hubner A, Bouillet JPD, Laclau JP, Nouvellon Y, Epron D (2013). Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. Forest Ecology and Management 301:6-27. http://dx.doi.org/10.1016/j.foreco.2012.12.030

Henriques EP, Moraes CB, Sebbenn AM, Tomazello Filho M, Moraes MLT, Mori ES (2017). Genetic parameters estimates for silvicultural traits and wood density in *Eucalyptus urophylla* progeny test. Scientia Forestalis 45(113):119-128.http://dx.doi.org/10.18671/scifor.v45n113.11

Brazilian Tree Industry (IBÁ) (2017). Annual Report IBÁ, 80p. Available in: https://iba.org/images/shared/Biblioteca/IBA_RelatorioAnual2017.pdf

Mato-Grossense Institute of Agricultural Economics (IMEA) (2013). Diagnóstico de Florestas Plantadas do Estado de Mato Grosso. Federation of Agriculture and Livestock of the State of Mato Grosso, 106p. Available in http://imea.com.br/upload/Relatorio_final_floresta_plantada.pdf

Kalil Filho NA, Pires CLS, Gurfinkel J (1982). Estimação de parâmetros genéticos e observação do comportamento em *Eucalyptus salign* Smith em Angatuba (SP). In: Congresso Anual da Associação Brasileira Técnica de Celulose e Papel – Semana do papel, São Paulo: ABTCP.

Lima JL, Souza JC, Ramalho MAP, Andrade HB, Sousa LC (2011). Early selection of parents and trees in Eucalyptus full-sib progeny tests. Crop Breeding and Applied Biotechnology 11(1):10-16. http://dx.doi.org/10.1590/S1984-70332011000100002

Marques Júnior OG (1995). Estimativas dos parâmetros genéticos e fenotípicos e avaliação da eficiência da seleção precoce em *Eucalyptus cloeziana* F. Muell. Master dissertation, Genetics and Plant Breeding, Federal University of Lavras, Lavras/MG, Brazil. 69p.

Massaro RAM, Bonine CAV, Scarpinati EA, Paula RC (2010). Early selection viability in *Eucalyptus* spp. clonal tests. Ciência Florestal 20(4):597-609. http://dx.doi.org/10.5902/198050982418

Moraes CB, De Freitas M, Casella T, Pieroni GB, Resende MDV, Zimbacks L, Mori ES (2014a). Genetic parameters of Eucalyptus clones for early selection in frost occurrence region. Scientia Forestalis 42(102):219-227. Available in: http://www.ipef.br/publicacoes/scientia/nr102/cap06.pdf

Moraes CB, Brizolla TF, Teixeira LG, Zimback L, Tambarussi EV, Chaves R, Mori ES (2014b). Estimates of genetic parameters for selection of trees in *Eucalyptus*. Scientia Forestalis 42(104):623-629. Available in: http://www.ipef.br/publicacoes/scientia/nr104/cap16.pdf

Moraes CB, Carvalho EV, Zimback L, Luz OSL, Pieroni GB, Mori ES, Leal TCAB (2015). Genetic variability of half sibs *Eucalyptus* progenies for cold tolerance. Revista Árvore 39(6):1047-1054. http://dx.doi.org/10.1590/0100-67622015000600007

Pavan BE, Paula RC, Perecin D, Scarpinati EA, Candido LS (2014). Early selection in open‑pollinated *Eucalyptus* families based on competition covariates. Pesquisa Agropecuária Brasileira 49(6):483-492. http://dx.doi.org/10.1590/S0100-204X2014000600010

Pinto DS, Resende RT, Mesquita AGG, Rosado AM, Cruz CD (2014). Early selection in tests for growth traits of *Eucalyptus urophylla* clonestest. Scientia Forestalis 42:251-257. Available in: http://www.ipef.br/publicacoes/scientia/nr102/cap09.pdf

Pires IE, Resende MDV, Silva RL, Resende Junior MFR (2011). Genética Florestal 1 ed. Editora Arka: Viçosa, 318p.

Resende MDV (2002). Software SELEGEN-REML/BLUP. Colombo: Embrapa Florestas. Documentos, 67p. Available in: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/43908/1/DC0077.pdf

Resende MDV, Duarte JB (2007). Precision and quality control in variety trials. Pesquisa Agropecuária Tropical 37:182-194. Available in: https://www.revistas.ufg.br/pat/article/download/1867/1773

Rocha MGB, Pires IE, Xavier A, Cruz CD, Rocha RB (2006). Genetic evaluation of half-sib *Eucalyptus urophylla* progenies by the REML/BLUP and minimum squares procedure. Ciência Florestal 16(4):369-379. Available in: https://core.ac.uk/download/pdf/25779827.pdf

Santos FW, Florsheim SMB, Lima IL, Tung ESC, Silva JM, Freitas MLM, Moraes MLT, Sebbenn AM (2008). Variação genética para a densidade básica da madeira e caracteres silviculturais em uma população base de *Eucalyptus camaldulensis* Dehnh. Revista do Instituto Florestal 20(2):185-194. Available in: http://www.iflorestal.sp.gov.br/RIF/RevistaIF/RIF20-2/RIF20-2_185-194.pdf

Tambarussi EV, Lima BM, Queiroz RC, Peres FSB, Dias DC, Pagliarini MK, Pereira FB, Rosa JRBF, Rezende GDSP (2017). Estimate of genetic parameters for early selection in clones of *Eucalyptus* spp. Scientia Forestalis 45(115):507-517. http://dx.doi.org/10.18671/scifor.v45n115.08

Vencovsky R, Barriga P (1992). Genética Biométrica no Fitomelhoramento. Ribeirão Preto: Revista Brasileira de Genética, 496p.

Wu HX (1998). Study of early selection in tree breeding: 1. Advantage of early selection through increase of selection intensity and reduction of field test size. Silvae Genetica 47(2-3):146-155. Available in: https://www.thuenen.de/media/institute/fg/PDF/Silvae_Genetica/1998/Vol._47_Heft_2-3/47_2-3_146.pdf

Wu HX (1999). Study of early selection in tree breeding: 2. Advantage of early selection through shortening of breeding cycle. Silvae Genetica 48(2):78-83.

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