

**Modern Trends in Conservation, Utilization
and Improvement of
Plant Genetic Resources**
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SEED VARIABILITY IN HIGH YIELDING *HEVEA BRASILIENSIS* CLONES

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Abstract: Natural rubber tree, *Hevea brasiliensis* Muell. Arg., primarily produces latex and its seeds are important feed stocks for non-edible oil production. Rubber seeds gained high economic importance due to its potential for the replacement of fossil fuel through biodiesel production. The present study deals with the assessment of trait variability of rubber seeds of recommended clones. Fresh seeds of newly released RRII 400 series clones viz., RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430 were collected from representative locations of traditional rubber growing areas of Kerala and Tamil Nadu. Seed morphological traits were recorded and their associations were estimated. The results demonstrated that the highest dry biomass was acquired by the seeds of clone RRII 414 (4.5 g) across locations, followed by RRII 417 (3.3g), RRII 429 (3.3g), RRII 430 (3.3g) and RRII 422 (3.0g). Correlation studies revealed direct relationship between total biomass and kernel weight of seeds. The highest kernel dry weight was registered by the clone RRII 414 (2.4g) followed by RRII 417(2.0g), RRII 429 (2.0g), RRII 430 (1.8g) and RRII 422(1.4g). RRII 429 registered the highest percentage of germination (67 - 80) compared to RRII 422 (52-65) and RRII 414 (55-64). This might be due to high kernel weight and reserve food availability in the kernels. Besides these traits, mottling pattern on the surface of seeds of each clone was found unique and visually stable, reconfirming its utilization for clone identification. The present study revealed that seed weight is a marker for high kernel biomass and this can be used for sorting seeds for commercial oil production and stock seedling plant generation.

Key Words: Rubber seed, alternate oil source, clonal variability

INTRODUCTION

Natural rubber tree, *Hevea brasiliensis* Muell. Arg., primarily produce latex with multiple utility in automobile tyre industry and it touches almost all fields of day-to-day life. Progress in yield improvement in *Hevea* resulted in a gradual increment up to about 2500 k/ha (Priyadarshan and Clement-Demange, 2004) through cyclic breeding and selection of elite genotypes. Systematic breeding and selection resulted in the generation of genotypes with earliness and high rubber yield. The exploitation of heterosis is a common objective in plant breeding (Mayo, 1987) and in rubber, testing of F_1 clonal progenies for growth, yield and other secondary traits like fruit bearing needs long evaluation trials. Recently, lengthy breeding cycle in *Hevea* was brought down considerably through clonal nursery approach (Gireesh *et al.*, 2017). Most of the new generation rubber clones are highly fertile and the seeds produced are either used for developing root stocks for propagation or unused. Only a small fraction of total production is being used as feed stocks for non-edible oil production which has not been thoroughly exploited. Rubber seeds have attained high economic importance and potential as an alternative oil source (Klopfenstein and Walker, 1983). Rubber seed oil can be used in additives or surface coatings (Aigbodiona and Pillai, 2000), cosmetics (Lourith *et al.*, 2014), biogasoline (Shuhaili, 2016) and edible oil replacement for biodiesel production (Huy *et al.*, 2014). Ever increasing environmental problems point towards

reduction of greenhouse gas emissions that warrants search for new renewable energy resources. Global biodiesel production is set to reach about 24 billion liters by 2017, and shortage of edible oil for human consumption in developing countries does not favour its use for biodiesel production (Divakara *et al.*, 2010). Recently, Rubber Research Institute of India (RRII) developed high yielding clones in RRII 400 series (Licy *et al.*, 2003 and Mydin *et al.* 2017), with improved potential of timber and biomass, apart from latex production. However, no information is available on seed traits of these clones. The present study deals with assessment of seed variability and interrelationship of seed traits among recommended rubber clones.

MATERIALS AND METHODS

Rubber seeds used in this study were collected from trees of similar age. Seeds of clones (RRII 414, RRII 417, RRII 422, RRII 429 and RRII 430) were brought to the laboratory, cleaned and fresh weight was determined. Traits observed were: length (mm); width (mm); thickness (mm); fresh weight (g); dry weight (g); volume (cc) and kernel weight (g) (Table 1). General view of seeds of different clones is illustrated in Fig. 1. Fresh seeds were germinated in seedling beds under shade and given sufficient water until germination. Extent of germination was scored up to seven days after sowing. Length, width, thickness and weight of randomly selected seeds ($n=50$) of each clone were measured using vernier calipers. Volume was estimated by water displacement method in graduated measuring cylinder and weight of each seed determined gravimetrically. Dry weight of the seeds was measured after drying in hot air oven at 60°C till the seeds attained constant weight. After drying, the seeds were deshelled manually using laboratory mortar and pestle and weight recorded (Table 2). Samples were stored in air tight polythene covers for further analysis. Statistical analysis was done (Singh and Chaudhary 1985); means and coefficient of variations were worked out.

RESULTS AND DISCUSSION

H. brasiliensis is a cross pollinated tree species with low level occurrence of self-pollination in the natural habitat. Flowers are monoecious in nature with male and female flowers emerging separately in the same inflorescence. Ratio of male to female flowers varies with different clones and natural seed setting amounts to 3-5 percent while some of the cultivated clones like PB 330 exhibit high rate of fruit set of about 15 percent (Chandrasekhar *et al.*, 2004). Latex production has commercial importance while secondary economic traits like seed production and its utilization have not gained importance. It is now being considered as safe alternative for edible oil resources for biodiesel production. In India, rubber cultivation occupies nearly eight lakh hectares mainly located in the state of Kerala and adjoining districts of Tamil Nadu and Karnataka. Commercial rubber cultivation is now being taken up in large scale by the North-Eastern states like Tripura, Meghalaya and Assam. But, only a very small fraction of seeds from commercial plantations is being exploited for oil extraction and stock seedling production. Fungal diseases play a major role in setting and sustenance of fruits until maturity.

Table 1. Seed traits in RR11 400 series clones

Clones	Length (mm)			Width (mm)			Thickness (mm)			Fresh wt. (g)			Volume (cc)			Dry wt. (g)			Kernel wt. (g)		
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
RR11 414	32.2	26-36	26.4	24-29	22.9	20-25	6.3	3.8-8.2	10.2	7-14	4.5	2.4									
RR11 417	25.8	20-33	22.2	19-26	19.8	17-22	4.3	2.8-7.0	7.6	5-12	3.3	1.9									
RR11 422	25.6	22-31	22.6	20-25	19.6	17-23	4.0	2.4-7.3	7.6	5-10	3.0	1.4									
RR11 429	26.5	22-33	22.9	19-27	20.3	17-20	4.8	2.4-7.6	8.4	7-12	3.3	2.0									
RR11 430	26.1	23-30	23.2	20-26	19.3	17-22	3.9	2.3-5.9	6.7	6-10	3.3	1.8									
CV	3.8	--	3.6	--	2.8	--	9.6	--	8.5	--	11.0	9.9									
SE	0.6	--	0.5	--	0.3	--	0.2	--	0.4	--	0.2	0.1									
CD	1.4	--	1.4	--	0.7	--	0.6	--	0.9	--	0.5	0.2									

Fig. 1. General view of fresh seeds of RR II 400 series clones; difference in seed mottling pattern is a distinct morphological marker for clone identification.



Table 2. Kernel biomass and germination of seeds

Clones	% of kernel dry wt.to fresh wt. of seeds	% of kernel dry wt.to dry wt. of seeds	Germination %	
			Range	Mean
RR II 414	37.7	52.9	55.0 - 64.0	58.0
RR II 417	44.6	57.3	55.0 - 60.0	57.5
RR II 422	34.2	45.7	52.0 - 65.0	59.5
RR II 429	40.8	59.6	66.7 - 80.0	72.0
RR II 430	45.4	53.9	54.0 - 62.0	59.6

In the present study, fresh seed weight from different clones varied from 3.9g to 6.3g across clones. The high yielding clone RR II 414 registered the highest volume and fresh seed weight (6.3g) followed by seeds of RR II 429 (4.8g), RR II 417 (4.3g) and RR II 422(4.0g). RR II 414 is found superior in terms of dry weight of seeds (4.5g) and kernel weight (2.4g)

followed by RR II 429 with 3.3g and 1.8g respectively. Seeds of RR II 430 are the smallest in size (Table 1). Successful introduction of high yielding clones sometimes contributes towards seed production also. Seed weight is one of the critical yield components and viability of seed is reported to be related to seed weight (Murali, 1997).

Rubber seeds may be one of the viable alternatives for non-edible oil production. As any other traits, variability of seed traits play an important role in the selection of good quality seed lot for oil production and selecting clones for stock seedling production among high yielding mother trees. In addition to this, difference in seed mottling pattern (Fig.1) is widely being used as a reliable distinct morphological marker for clone identification, as the mottling pattern might be controlled by very few genes. Quantity of stored food materials in the heavy seeds might have contributed to early and fast germination (Banik, 1978). Interrelationship among different seed traits (Table 3) shows that seed weight is highly correlated with kernel weight. On an average, healthy rubber tree can set about 500g of useful seeds during a normal flowering season with less prevalence of fungal diseases. Generally 37 percent by weight of the seed biomass is shell and the rest is kernel (Atabani *et al.*, 2013). However, it is assumed that heavier seed might hold more oil content thereby screening seeds for weight are rather easy. Therefore, using seed weight as a marker trait could enhance selection efficiency of seeds *viz-a-viz* recovery of quality seedlings for stock plants production. In the present study, seed traits expressed by RR II 414, RR II 429 and RR II 430 are superior. Ponnammal *et al.* (1993) reported that biomass of seedlings increased with increase in seed size and seed weight in plants like *Hardwickia binata*. Present observations suggest that RR II 414 is a potential parent clone for both improving seed yield as well as rubber yield and its mating with PB 330 may be expected to increase seed setting rate in progenies.

A study conducted by Aparicio *et al.* (2002) says that the surface area of the seed and the area of the embryo determined the seed traits and influenced by changing seed grading size. However, since the two variables were strongly correlated, it was not possible to separate their relative contribution to the development and growth of seedlings. Only 60 percent of the seeds of RR II 430 were germinated (59.6 percent) despite its high kernel weight, 54.9 percent of dry weight of seeds. Whereas, highest germination percentage was observed from the clone RR II 429 with a range of 67- 80 percent, wherein percentage of kernel dry weight to dry weight of the seedling was 60% (Table 2). Relationship between different seed morphological traits is illustrated in the Table 3. Dry weight of seeds found highly related with the dry kernel weight. Interrelationships among the morphological traits also play a major role in selecting seeds for successful stock plant production for commercial nurseries. In certain tree species, there was significant correlation between seed character and seedling growth attributes as in the case of *Anacardium occidentale* (Anjusha *et al.* 2015). Percentage of germination found direct relationship with recovery of higher number of hybrid seedlings consequent to natural and artificial hybridizations. Economic gain from breeding and propagation programmes largely depending on the extent of viability and germinability of seeds from the mother trees. The high germination rate and faster growth of the seedling may be attributed to greater food reserves available to the growing seedling (Yusuf *et al.*

2014). Seeds with higher biomass and kernel weight could generate healthy seedlings and thereby augments the chances of better selection.

Table 3. Interrelationship between different seed traits.

	Length (mm)	Width (mm)	Thickness (mm)	Fresh wt. (g)	Volume (cc)	Dry wt. (g)	Kernel wt. (g)
Length (mm)	1.00						
Width (mm)	0.89	1.00					
Thickness (mm)	0.94	0.87	1.00				
Fresh weight (g)	0.76	0.69	0.79	1.00			
Volume (cc)	0.81	0.71	0.86	0.59	1.00		
Dry wt. (g)	0.77	0.63	0.71	0.53	0.56	1.00	
Kernel wt. (g)	0.68	0.64	0.67	0.55	0.48	0.80	1.00

At present, rubber seed oil industry and stock seedling production are mainly based on the two popular cultivated clones like RRIM 600 and RR II 105. However, extend of these clones has been reduced considerably during the recent past. Therefore, identifying alternate seed bearing clones for quality seed production is essential to meet the future demand. Genetic improvement programmes have resulted in the development of productive clones and its potential to produce quality seeds is also established. Therefore, it is also important to evaluate clones for their level of fertility/fecundity and seed bearing capacity in rubber growing tracts. The present study is also suggestive of initiating tree improvement using conventional breeding strategies incorporating highly fertile parental clones.

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