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Physiological Quality and Desiccation Sensitivity of Rubber (*Hevea brasiliensis)* Seeds during Fruit Maturation

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ABSTRACT

The physiological quality and desiccation of rubber seeds were investigated during development. Fruits of rubber clone RRIM 600 at different stages of development were collected from a plantation in Nakhon Sri Thammarat province, southern Thailand, during July–August 2012. Seeds of deep green fruit, yellowish-green fruit, brown fruit and from the naturally shedding stage were tested for their weight, moisture content, germination, mean germination time, seedling shoot height, seedling shoot dry weight, electrical conductivity and desiccation tolerance during dry storage. Rubber seeds with high germination ranging from 86.67 to 95.00% were obtained from the yellowish-green fruit, brown fruit and naturally shedding stages. However, the seeds reached physiological maturity at the brown fruit stage with values for seed dry weight, moisture content and germination of 2.93 g per seed, 34.54% and 95.00%, respectively. At the shedding stage, the seeds had a moisture content and a high germination of 22.17% and 91.66%, respectively. The critical moisture content of the seeds was 15% and at this level, they were very short lived (3–7 d in dry conditions). The seeds with a moisture content of about 15% and from 11 to 12% had germination of 58.33–63.34% and 1.67–8.34%, respectively. Therefore, the early collection of the shed seed is recommended to ensure high quality seed is obtained. Such information may be helpful for improving the seed collection or transport procedures and ensuring high seed performance in the seedbed for successful rootstock production.

Keywords: rubber (*Hevea brasiliensis*), seed quality, recalcitrant seed, maturity, seed desiccation

INTRODUCTION

Rubber (*Hevea brasiliensis*) is an important economic commodity. Most rubber plantations are planted with budded seedlings or a budded rubber stump either in polyethylene bags or as bare-rooted stumps obtained by grafting a scion of a high yielding clone onto a seedling rootstock (Rubber Research Institute of Thailand, 2013). The production of budded stumps normally requires a lot of seed for rootstock production. Rubber Research Institute of Thailand (2013) reported that the area of rubber plantation and the replanted areas of Thailand in 2010 were about 2,931,201 and 34,228 ha, respectively which required budding of more than 17,114,000 seedlings for the replanting operation. Therefore, a high quantity of rubber seed is needed for rootstock production each year. The success of rootstock production with rubber trees largely depends on the availability of good quality seed from which to produce good quality saplings that are uniform,

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healthy and vigorous, which in turn influences the budding process and also the rootstock's influence on the performance of the scion after the budding (Daud *et al*., 2012). However, it is often found that rubber seed sourced from natural shedding has low quality, which might be due to the rapid loss of viability after shedding under natural conditions because of the recalcitrant behavior of the seeds by the desiccation sensitivity (Chin and Roberts, 1980). The seed maturity and the moisture content are crucial in determining seed viability (Lan *et al*., 2007). Kaushik (2003) reported that the vigor of *Jatropha curcas* seeds obtained from green fruit was lower than that from yellow or brownish-black fruits and the best physiological quality seeds were obtained from yellow fruit. During desiccation, seeds of *Hopea parviflora* and *H. pomga* reduced germination below a moisture content of 26% and 28%, respectively (Rajeeswari and Kaveriappa, 2000). Seed of *Mangifera persiciformis* lost 50% viability at a moisture content of 21.50% and seed with a moisture content of 18.3% had almost no germination (Tang *et al*., 2008). However, there is a lack of sufficient knowledge of rubber seed maturity and desiccation after shedding for quality control and management to ensure the use of high quality seed for rubber root stock production.

The objectives of this investigation were to study rubber seed quality during fruit maturation and the change in seed quality during the desiccation of the rubber seed.

MATERIALS AND METHODS

Collection of fruits

Fruits of rubber clone RRIM 600 at different stages of development were collected from a plantation in Nakhon Sri Thammarat province, southern Thailand, during July–August 2012. Seeds from four developmental stages: 1) immature fruit (deep green fruit), 2) early mature fruit (yellowish-green fruit), 3) mature fruit before shedding (brown fruit with a breaking strip on the pericarp) and 4) fruit-shedding stage (Figure 1) were tested for quality in four replicates.

Moisture content and fresh and dry weight

The moisture content was determined for whole seed, the embryo, endosperm and testa. Six seeds and 10 pieces of each structure per replication were weighed and dried at 105 ^{o}C for 24 hr. Dry seed and its structure were weighed, and the moisture content was calculated on a percentage of wet weight basis. The fresh weights of whole seed and the seed parts were also calculated in grams per seed.

Germination

Fifteen seeds per replication were germinated in moist sand in a plastic basket and placed in plastic lathe house. First and final counts were done at 7 and 21 d after germination (DAG), respectively (Chin and Roberts, 1980). Normal seedlings were averaged as the germination percentage.

Mean germination time

The seeds were germinated in a germination test. Based on the rubber seed germination behavior, normal seedlings were counted at 7, 14, and 21 DAG and the mean germination time (GMT) was calculated using the formula in Equation MGT = $\Sigma nd / \Sigma n$ as described by Ellis and Roberts (1981):

Where n is the number of seeds which germinate on day D and D is the number of days counted from the beginning of the test.

Seedling growth

Five normal seedlings per replication from the germination test were measured for shoot length. The seedling shoots were cut above the sand-medium surface and the shoots from each replication were separately dried at 80 °C for 24 hr. Dry seedling shoots were weighed.

Electrical conductivity

Five seeds per replication were weighed

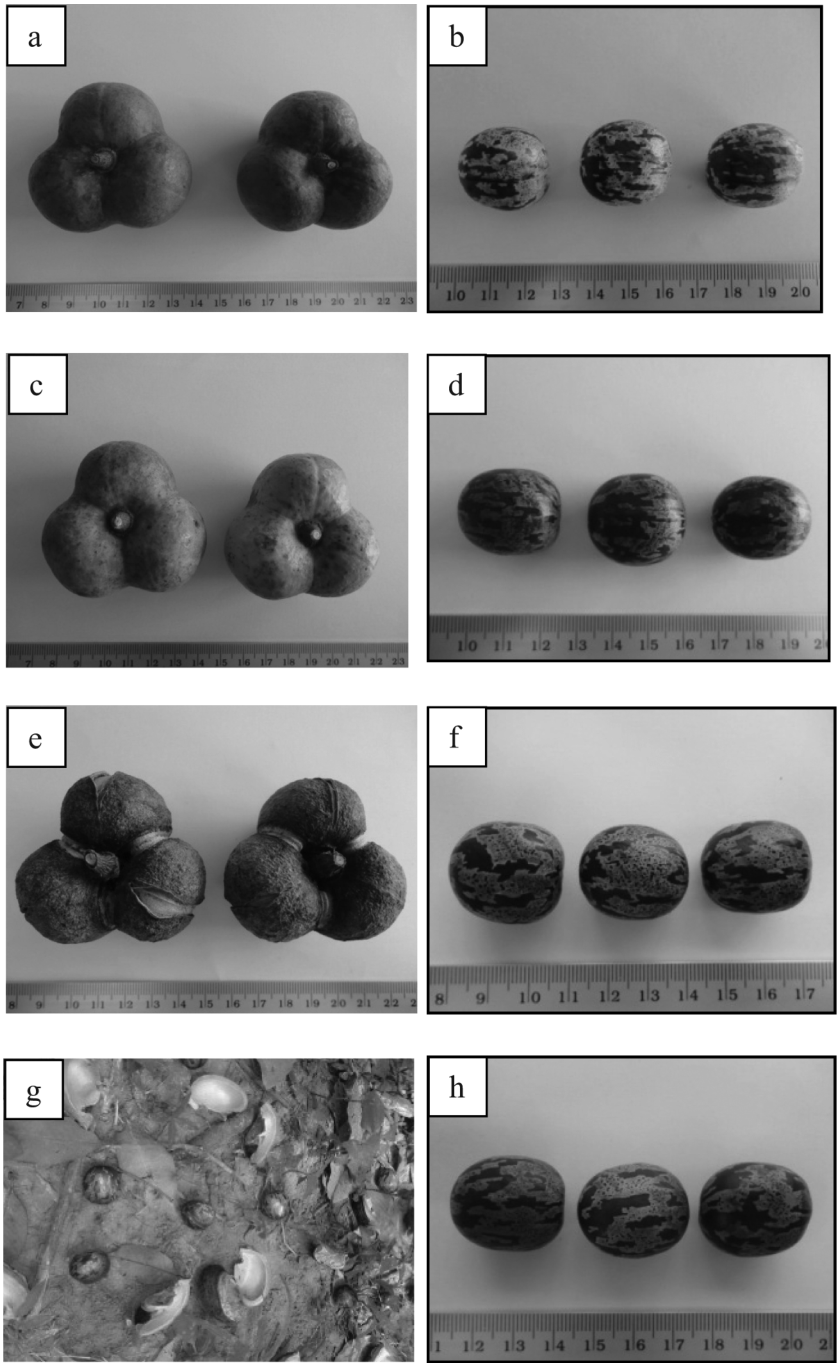

Figure 1 Rubber fruit and seed at different stages of development: (a) Deep green fruit; (b) Seed from deep green fruit; (c) Yellowish-green fruit; (d) Seed from yellowish-green fruit; (e) Brown fruit; (f) Seed from brown fruit; (g) Naturally shedding fruit; (h) Seed from naturally shedding fruit.

and soaked in 75 mL of deionised water at 25 °C for 24 hr. The electrical conductivity of the steep water was measured using a conductivity meter.

Desiccation tolerance and storability

Seeds from the different sampled fruit developmental stages were stored in plastic baskets at room temperature for 0, 3 and 7 d. Then, the stored seeds were tested for their moisture content, germination, mean germination time, seedling shoot dry weight and electrical conductivity using the procedures described earlier.

Statistical analysis

The experiments were arranged in a completely randomized design and their means were compared using Duncan's multiple range test.

RESULTS

Quality of rubber seed at different developmental stages

The fresh and dry weights of rubber seed and its parts corresponded to their development or maturity. The changes in the fresh weight and dry weight of rubber seed and its structures throughout development are shown in Table 1. The fresh weight of the whole seed, embryo, endosperm and testa gradually decreased from the green fruit to the naturally shedding stage with values of 4.94–3.47, 0.31–0.27, 2.39–1.73 and 1.63–1.37 g per seed, respectively. The dry weights of the whole seed, embryo, endosperm and testa exhibited similar changes with a gradual increase during the early stage of development, reaching a maximum at the brown fruit stage, followed by a slight decrease. From the green fruit to brown fruit stage, the seed dry weight increased from 2.34 g per seed to a maximum of 2.93 g per seed and then declined to 2.69 g per seed by the natural shedding stage. The changes in the embryo, endosperm and testa dry weights corresponded with that reported for the whole seed. The dry weight of the embryo, endosperm and testa were maximal during the brown fruit stage with values of 0.22, 1.41 and 1.30 g per seed, respectively.

The moisture content of the seed and the embryo, endosperm and testa, gradually declined from the deep green fruit to the naturally shedding stage. The seed moisture content decreased with maturation from 52.52% to 22.17% which was statistically significant ($P < 0.01$). The change in the moisture content of the embryo, endosperm and testa during development corresponded with that in the whole seed. At the naturally shedding stage, the moisture content of the embryo, endosperm and testa were minimal with values of 23.82, 23.78 and 12.83%, respectively (Table 2).

The germination and vigor of rubber seed depended on the stage of development. Seed from deep green fruits had the minimal germination

Table 1 Fresh and dry weights of rubber seed and its parts in different fruit developmental stages.

Fruit developmental stage	Fresh weight (g per seed)				Dry weight (g per seed)			
	Whole seed	Embryo	Endosperm	Testa	Whole seed	Embryo	Endosperm	Testa
Deep green fruit	4.94^{a}	0.31^{b}	2.39^{a}	1.63^{a}	2.34^{c}	0.15^{b}	0.88^{d}	1.22^{bc}
Yellowish-green fruit	4.61^{ab}	0.32^{b}	2.50^{a}	1.55^{ab}	2.68^{b}	0.17^{b}	1.19^{c}	1.27^{ab}
Brown fruit	4.48^{b}	0.37^{a}	2.35^{a}	1.54^{b}	2.93^{a}	0.22^{a}	1.41^{a}	1.30^{a}
Naturally Shedding	3.47^{c}	0.27^{c}	1.73^{b}	1.37^{c}	2.69^{b}	0.21^{a}	1.32^{b}	1.20^{c}
F-test	*	*	**	*	**	**	**	*
CV (%)	6.14	6.81	5.06	3.72	3.72	8.41	4.84	3.73

Means in each column with the same lowercase letter are not significantly different by Duncan's multiple range test.
*,**Significant at $P < 0.05$ and $P < 0.01$, respectively; CV = Coefficient of variation.

Table 2 Moisture content of rubber seed and its parts in different fruit developmental stages.

Fruit developmental stage	Moisture content (%)			
	Whole seed	Embryo	Endosperm	Testa
Deep green fruit	52.52[a]	50.52[a]	63.22[a]	25.03[a]
Yellowish-green fruit	41.70[b]	48.37[a]	52.59[b]	17.74[b]
Brown fruit	34.54[c]	41.08[b]	40.03[c]	15.38[c]
Naturally shedding	22.17[d]	23.82[c]	23.78[d]	12.83[d]
F-test	**	**	**	**
CV (%)	7.00	7.57	5.33	3.46

Means in each column with the same lowercase letter are not significantly different by Duncan's multiple range test.
** Significant at $P < 0.01$; CV = Coefficient of variation.

of 16.67%. The germination did not change significantly after the yellow green fruit stage up to the naturally shedding stage with values in the range 86.67–95–91.66% (Table 3). The development of seed vigor in terms of the mean germination time, seedling shoot height, seedling shoot dry weight and electrical conductivity also showed similar trends to that of germination. The seed vigor increased as the fruit developmental stage increased and reached its maximum in the brown fruit stage, followed by a slight decrease in the naturally shedding stage. The mean seed germination time was high (21.00 d) prior to the yellowish-green fruit stage and then decreased to 14.35 d at the brown fruit stage, followed by a significant ($P < 0.05$) increase (18.21 d) for natural shedding seed. Seed from deep green fruits had a seedling shoot height of 9.33 cm which then increased to 37.95–35.35 cm in the later fruit developmental stages. Brown fruit seed produced the maximum seedling shoot dry weight of 0.62 g per seed, while seed from deep green fruits, yellowish-green fruits and the naturally shedding stage all produced lower seedling shoot dry weights of 0.07, 0.51 and 0.46 g per seed, respectively. The electrical conductivity of seed declined with the advancement of maturity from 2.14 $\mu S.cm^{-1}.g^{-1}$ at the deep green fruit stage to 1.09 $\mu S.cm^{-1}.g^{-1}$ at the brown fruit stage. The electrical conductivity significantly ($P < 0.01$) increased at the naturally shedding stage (1.39 $\mu S.cm^{-1}.g^{-1}$).

Table 3 Germination, mean germination time, seedling shoot height, seedling shoot dry weight and electrical conductivity of rubber seed from different fruit developmental stages.

Fruit developmental stage	Germination (%)	Mean germination time (d)	Seedling shoot height (cm)	Seedling shoot dry weight (g per seedling)	Electrical conductivity ($\mu S.cm^{-1}.g^{-1}$)
Deep green fruit	16.67[b]	21.00[a]	9.33[b]	0.07[c]	2.14[a]
Yellowish-green fruit	86.67[a]	17.18[b]	37.95[a]	0.51[b]	1.45[b]
Brown fruit	95.00[a]	14.35[c]	36.30[a]	0.62[a]	1.09[c]
Naturally shedding	91.66[a]	18.21[b]	35.35[a]	0.46[b]	1.39[b]
F-test	**	*	**	**	**
CV (%)	17.30	8.79	10.47	11.39	7.28

Means in each column with the same lowercase letter are not significantly different by Duncan's multiple range test.
*,**Significant at $P < 0.05$ and $P < 0.01$, respectively; CV = Coefficient of variation.

Changes of quality during desiccation of rubber seed of different developmental stages

The moisture content of seed declined with the maturation and desiccation period. Seeds obtained from the deep green fruit, yellowish-green fruit, brown fruit and the naturally shedding stages had moisture contents of 52.52, 41.70, 34.54 and 22.17%, respectively (Figure 2). At 3 d of desiccation, the moisture content of seed from deep green fruits was high (40.60%), while seeds from yellowish-green fruits, brown fruits and the naturally shedding stage had moisture contents of 26.37, 21.95 and 14.24%, respectively. Seeds of deep green fruits and all three stages of development after 7 d of desiccation dried to 30.94% and 15.30–10.74% moisture content, respectively.

The desiccation sensitivity of rubber seed depended on the development stage and dehydration times. Freshly harvested seed of deep green fruits had the lowest germination of 16.67% with seeds from the later stages of fruit development all producing the same germination in the range 86.67–95.00% (Figure 3). After 3 d

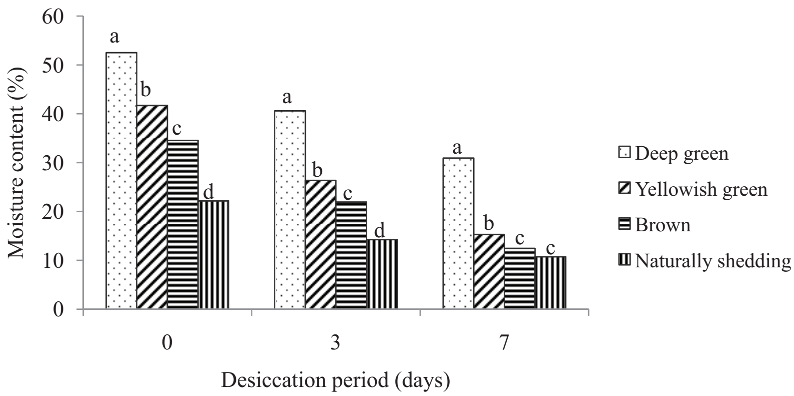

Figure 2 Moisture content of rubber seeds from different fruit developmental stages at different periods of desiccation. Fruit developmental stages in the same desiccation period with the same lowercase letter are not significantly different ($P < 0.05$).

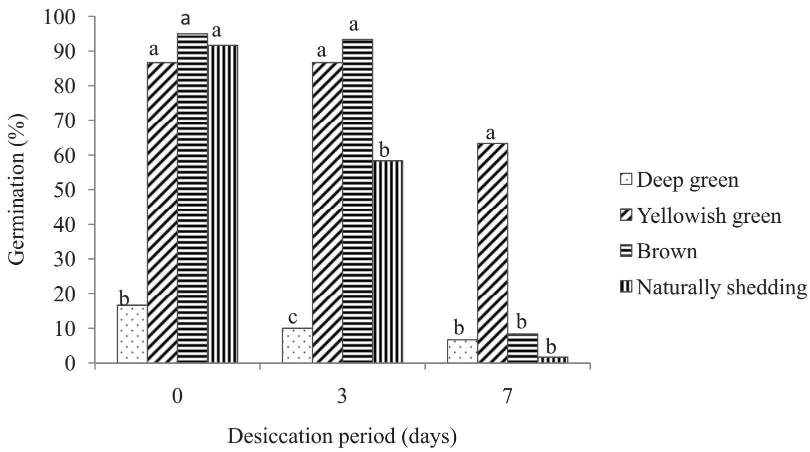

Figure 3 Germination of rubber seeds from different fruit developmental stages at different periods of desiccation. Fruit developmental stages in the same desiccation period with the same lowercase letter are not significantly different ($P < 0.01$).

of desiccation, the germination of seeds of deep green fruits and the naturally shedding stage was much lower than for seeds from the yellowish-green and brown fruit stages. At 7 d of desiccation, most seeds subsequently became more sensitive to desiccation with germination lower than 10%. However, the seeds obtained from yellowish-green fruits still had a higher germination of 63.34%.

The mean germination time and seedling shoot dry weight corresponded to the germination results (Figure 4 and 5). Seeds of yellowish-green fruit exhibited greater desiccation tolerance than those of the other stages at 7 d after desiccation. During desiccation, the electrical conductivity increased in every development stage, but the electrical conductivity of seed from deep

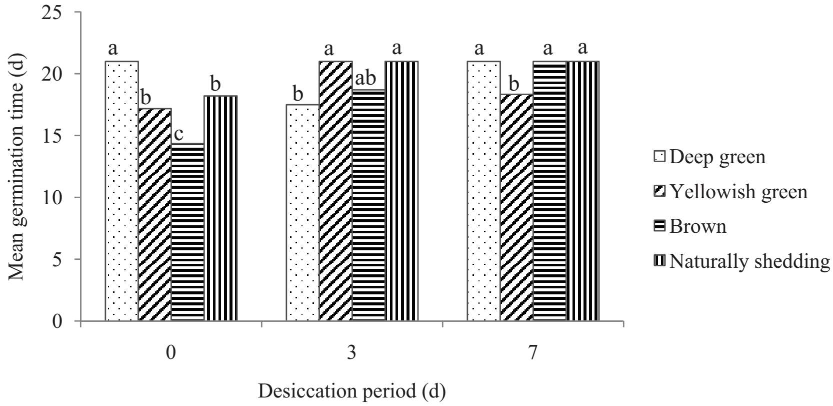

Figure 4 Mean germination time of rubber seeds from different developmental stages at different periods of desiccation. Fruit developmental stages in the same desiccation period with the same lowercase letter are not significantly different ($P < 0.05$).

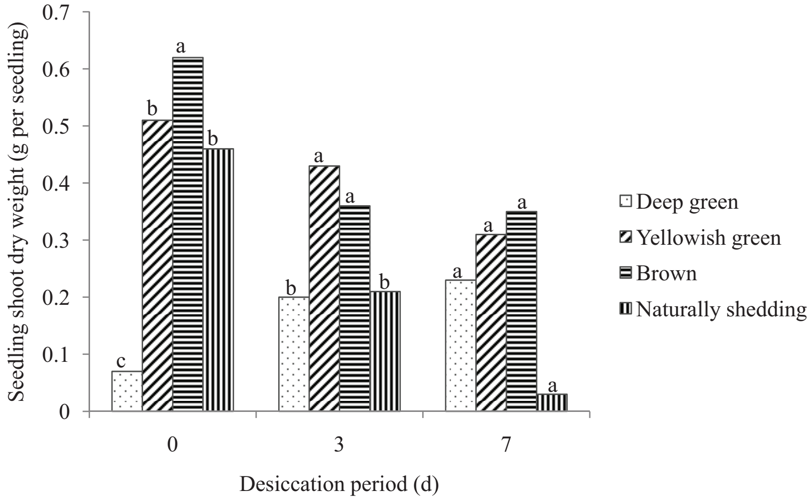

Figure 5 Seedling shoot dry weight of rubber seeds from different fruit developmental stages at different periods of desiccation. Fruit developmental stages in the same desiccation period with the same lowercase letter are not significantly different ($P < 0.01$ and 0.05 for 0 and 3 d of desiccation, respectively).

green fruits increased more rapidly than those of yellowish-green fruits, brown fruits and the naturally shedding stage (Figure 6).

DISCUSSION

Physiological maturity is the stage of development when the seeds reach their maximum dry weight and marks the end of seed-filling time (Lan *et al*., 2007). This point can be defined in many species using the number of days after anthesis or flowering such as for *Hopea hainanensis* (Lan *et al*., 2007) and *Artocarpus heterophyllus* (Chaudhury and Malik, 2004). For example, the physiological maturity of *Hopea hainanensis* seed appears to be at 157 d after anthesis, when the seed has attained its maximum dry weight of 0.43 g. However, there are exceptions to this; the physiological maturity stage of *Jatropha curcas* could be determined by the visual aspect of fruit color (Dranski Jr. *et al*., 2010; Silva *et al*., 2012). Physic nut seeds with high physiological quality are obtained from yellowish-brown fruits which gave the highest germination and vigor in terms of the first count of germination, accelerated aging and electrical conductivity (Silva *et al*., 2012). As shown in Table 1, rubber seed and its embryo, endosperm and testa, reach their maximum dry weight values of 2.93, 0.22, 1.41 and 1.30 g per seed, respectively, at the brown fruit stage. At this stage, the seeds had the highest physiological quality in terms of germination (95.00%), mean germination time (14.35 d), seedling shoot height (36.30 cm), seedling shoot dry weight (0.62 g per seedling) and electrical conductivity (1.09 $\mu S.cm^{-1}.g^{-1}$) as shown in Table 3. Seed of naturally shedding fruits had a similar germination and vigor as seeds of brown fruit although there was a slight decrease in the seedling shoot dry weight and electrical conductivity which indicated the deterioration of membrane integrity. Seed of yellowish-green fruits produced the same level of germination as that of brown fruits; however, lower seed vigor was found. The seeds from deep green fruits had a low germination capacity because of the low food accumulation (dry weight; 20.4 g per seed) as shown in Table 1 and there was incomplete membrane organization with the highest electrical conductivity of 2.14 $\mu S.cm^{-1}.g^{-1}$ (Table 3). Silva *et al*. (2012) found that physic nut seed obtained

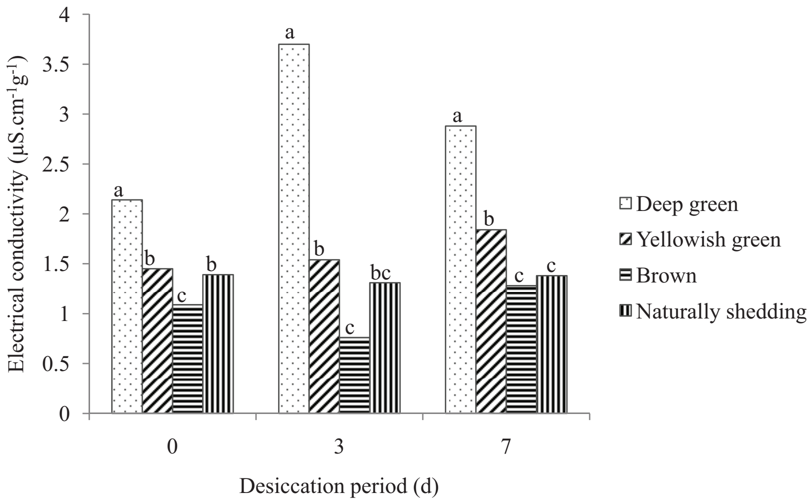

Figure 6 Electrical conductivity of rubber seeds from different fruit developmental stages at different periods of desiccation. Fruit developmental stages in the same desiccation period with the same lowercase letter are not significantly different ($P < 0.01$, 0.05 and 0.01 for 0, 3 and 7 d of desiccation, respectively).

from green fruits had the lowest physiological quality with the highest electrical conductivity of 113.95 μS.cm^{-1}.g^{-1}. The results of the current study indicated that during seed development and maturation, there were changes in the fresh weight, dry weight, moisture content, germination and vigor as described by Bewley and Black (1994). The rubber seed achieved physiological maturity at the brown fruit stage with the highest seed dry weight and germination potential.

The rubber seed lost viability rapidly within a few days under dry conditions (Figures 3, 4, 5 and 6). The data indicated that the rate of rubber seed deterioration depended on the maturation stage and the seed moisture content after desiccation. The seeds from the yellowish-green, brown and naturally shedding stages with moisture contents in the range 22.17–41.70% had high germination of 86.67–95.00% at 0–3 d of desiccation. The seeds with a moisture content of about 15% and 11% had germination of 58.33–63.34% and 1.67–8.34%, respectively, after 3–7 d of desiccation. The results corresponded with those of other researchers and other desiccation-sensitive species which exhibit a loss of viability below a critical moisture content of: 15–20% in rubber (Chin *et al*., 1981); 24% in mangosteen (Normah *et al*., 1997); and 40, 25 and 33% in mango, longan and lychee, respectively, (Fu *et al*., 1990). However, during the desiccation period, seed from the deep green fruits which was immature, had a low germination (16.67–6.67%) despite a high moisture content in the range 31.03–52.59%. The results indicated that the mature seed of any fruit stage (yellowish-green, brown and from natural shedding) exhibited no desiccation tolerance when the moisture content declined to 15% or below. In most seed, there was a noticeable loss in viability or germination performance. However, seed from the yellowish-green fruit with a moisture content of 41.70% after harvesting still had a moisture content of 15.30% with germination of 63.34% at 7 d after desiccation while those of the other mature fruit stages had moisture contents of about 11% with germination of only 1.67–8.34%. Lan *et al*. (2007) reported that *Hopea hainanensis* seed with an embryonic axis 150 d after anthesis exhibited the highest desiccation tolerance while the seed reached its physiological maturity at 157 d after anthesis. The results obtained from the collection of naturally shedding seed suggested that it should be collected as quickly as possible to ensure high seed quality. Any delay in the collection would result in increased levels of mortality and reduced quality. There would also be a cost associated with planting material, transportation, seedbed management as well as a possible adverse impact on seedling performance and cultivation practice in rootstock production. For the successful development of a breeding program and a future rubber rootstock seed garden where there is control over the canopy or height of the rubber trees, the optimal stage of fruit development should determine the timing of seed collection.

CONCLUSION

Rubber seeds with high physiological quality, germination and vigor were obtained from the yellowish-green stage, from the brown fruit stage and from the naturally shedding stage. The seed reached physiological maturity at the brown fruit stage with a seed dry weight, moisture content and germination of 2.93 g per seed, 34.549% and 95.00%, respectively. At the shedding stage, the seed had a moisture content and germination of 22.17% and 91.66%, respectively. However, the seed were very short-lived and had deteriorated within 3–7 d of dry conditions. The seeds with a moisture content of about 15% and 11–12% had germination of 58.33–63.34 and 1.67–8.34%, respectively. Therefore, it is suggested that newly shed seed needs to be collected as soon as possible to ensure high quality seed is obtained.

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