UNIVERSITI PUTRA MALAYSIA

SOYBEAN [Glycine max (L.) Merrill.] SEED QUALITY UNDER DIFFERENT PRODUCTION ENVIRONMENTS, STORAGE CONDITIONS AND SEED INVIGORATION USING HUMIC ACID

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Faculty : Agriculture

The government of Sri Lanka has targeted to increase the production of soybean by the area cultivated. This target was unable to be achieved due to lack of supply in good quality seeds at time of planting. Currently, seed production is only carried out in a single location in Sri Lanka, hence new areas for seed production are required. Seed quality is influenced by both the pre-and post-harvest factors. However, effect of pre harvest production environments on seed quality has not yet been clarified. Therefore, the aim of the first study was to determine the effect of seed production environment on seed development, maturation, and subsequent seed quality. The experiment was conducted at six production environments, three locations [Alutarama (ALU), Polonnaruwa (POL) Mahailuppalama (MI)] over two planting cycles (P1, P2). Seed development and maturation, seed and seedling quality characteristics were evaluated at five reproductive (R6, R7, R8, R8+5 and R8+10) maturity stages. The study infers that production environment at the late reproductive (LR) stage (R6-R8) was critical in determining the seed quality. If the LR coincides with more than 100 mm of rainfall or 75% of relative humidity considered as wet environment, otherwise considered as dry. A longer duration was required for the seeds to mature, around 27.5 days, if the LR stage coincided with wet environment (P1 ALU, P2 ALU P2 POL), in contrast to only 17.5 days in dry environment (P1 MI, P1 POL, P2 MI). Seed lots from dry environment during LR stage surpassed the minimum quality standards (75% final germination) at maturity stage R7 onwards, while this only occurred at maturity stage R8 for wet environments. Soybean seeds with minimum quality standards can be produced in all three locations provided harvesting is done at the correct LR stage. However, the quality of the seeds upon storage may differ due to the influence of the production environment. In the second experiment, full mature stage (R8) seeds were obtained from six environments, and upon drying, seeds were stored over one-year period in two different conditions, ambient (24.6 - 33.8 °C, RH 37-88%) and modified storage condition (20 °C ± 2°C, RH 60-70%). The quality of the stored seeds was evaluated at different storage periods (0, 2, 4, 6, 8, 10, 12 months) to monitor the rate of deterioration. Seeds were subjected to germination and vigor test, antioxidant enzyme activities, and malondialdehyde content. Seed lots that matured in dry environments maintained minimum quality standards for over 12 months under modified storage conditions while only 8 months of storage was possible in ambient condition. A slower rate of reduction was obtained in antioxidant capacity for seeds stored under modified conditions compared with ambient. Thus, modified store is recommended with comparatively low construction (1/3) and running (1/4) cost compared with cold storage. Despite the improvement in storability, decline in seed vigour during storage is inevitable due to ageing. The ageing effects can be alleviated, through seed priming. Thus, the third experiment investigated the effects of Humic acid (HA) (an environmentally safe priming agent) on activities of antioxidant enzymes and lipid peroxidation along with the consequences on seed germination in naturally aged soybean seed. Seed lot that was removed from a 12-month storage under ambient condition with 60±5% germination was primed using distilled water or 0.2 g/L HA solution at 25 °C for 1, 3, 5 and 7 h with non-primed dry seeds as control, resulting in nine treatment combinations. Laboratory and field experiments were carried out to test for the best priming treatment to improve seed germination and seedling vigour. HA priming for 5h mitigated the adverse effect of seed deterioration on germination-related traits by increasing the antioxidant enzyme activity [catalase (CAT) and peroxidase (POD)] and reducing malondialdehyde (MDA) content. In conclusion, soybean seeds can be produced above the minimum quality standard in P1 at MI and POL by managing planting time (from January to March and in P2 at ALU, POL and MI from June to August and can be stored for 8 months under modified storage condition. Overall findings from this study can be used to increase soybean seed availability at time of planting.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KUALITI BIJI BENIH KACANG SOYA [*Glycine max* (L.) Merrill.] DI BAWAH PERSEKITARAN PENGELUARAN YANG BERBEZA, KEADAAN PENYIMPANAN DAN PENCERGASAN BIJI BENIH MENGGUNAKAN ASID HUMIC

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Kerajaan Sri Lanka menyasarkan untuk mempertingkatkan pengeluaran dan meluaskan penanaman kacang soya atas dasar keselamatan makanan dan makanan ternakan negara tetapi sukar dicapai kerana menghadapi masalah pengeluaran biji benih yang berkualiti. Ketika ini, pengeluaran biji benih hanya dijalankan di satu kawasan sahaja. Oleh itu, kawasan baharu diperlukan untuk meningkatkan pengeluaran biji benih. Faktor pra dan pasca tuai banyak mempengaruhi kualiti biji benih. Oleh itu, kajian pertama telah dilakukan untuk menentukan kesan persekitaran semasa penanaman biji benih terhadap perkembangan benih, kematangan dan seterusnya kualiti biji benih yang dihasilkan. Eksperimen dilakukan di enam persekitaran yang berlainan iaitu tiga kawasan yang berbeza [Alutarama (ALU), Polonnaruwa (POL) dan Mahailuppalama (MI)] dan dua kitaran penanaman (P1 dan P2). Perkembangan dan kematangan serta kualiti biji benih dinilai mengikut lima peringkat kematangan (R6, R7, R8, R8+5 dan R8+10). Kajian menunjukkan persekitaran penanaman di peringkat akhir kematangan (R6-R8) merupakan peringkat yang penting dalam menentukan kualiti biji benih kacang soya. Jika peringkat akhir kematangan berlaku pada musim lembap (P1 ALU, P2 ALU P2 POL), kematangan mengambil masa yang lama iaitu sekitar 27.5 hari berbanding semasa musim kering (P1 MI, P1 POL, P2 MI) iaitu sekitar 17.5 hari. Biji benih yang dituai semasa peringkat akhir kematangan semasa musim kering melepasi minimum biji benih berkualiti (percambahan melebihi 75%) dan ini dicapai sekitar peringkat kematangan R7 berbanding semasa musim lembap iaitu pada peringkat kematangan R8. Biji benih kacang soya berkualiti dapat dihasilkan di ketiga-tiga lokasi, dengan syarat penuaian dilakukan di peringkat kematangan yang betul. Akan tetapi, kualiti biji benih semasa penyimpanan mungkin berbeza akibat pengaruh kesan persekitaran. Eksperimen kedua dijalankan dengan menyimpan biji benih yang dituai pada peringkat kematangan R8 selama setahun di dua keadaan yang berbeza iaitu penyimpanan suhu bilik (suhu 24.6 – 33.8 °C, kadar kelembapan 37-88%) dan dalam keadaan penyimpanan yang dimodifikasi (suhu 20 ± 2°C, kadar kelembapan 60-70%). Kualiti biji benih dinilai pada setiap 2 bulan sepanjang tempoh penyimpanan. Ujian seperti ujian percambahan dan kesegahan benih, ujian konduksi elektrik, aktiviti enzim anti-oksida, dan jumlah malondialdehid dilakukan sepanjang tempoh tersebut. Hasil ujian menunjukkan biji benih yang di simpan dalam penyimpanan dimodifikasi dapat mengekalkan minimum kualiti biji benih sepanjang tempoh 12 bulan berbanding biji benih yang disimpan dalam penyimpanan suhu bilik di mana kualiti menurun selepas 8 bulan. Hasil ujian menunjukkan anti-oksida biji benih lebih tinggi apabila ianya disimpan di ruang penyimpanan dimodifikasi berbanding penyimpanan suhu bilik. Oleh itu penyimpanan dalam keadaan suhu 20 °C ± 2°C, kadar kelembapan 60-70% boleh dilakukan sebagai alternatif penyimpanan biji benih kacang soya kerana kos pembinaan (1/3) dan kos penyelenggaraan (1/4) yang lebih murah berbanding penyimpanan bersuhu rendah. Walau bagaimanapun, kesegahan biji benih kacang soya menurun sepanjang tempoh menyimpanan akibat penuaan. Kesan penuaan dapat dikurangkan melalui kaedah priming biji benih menggunakan pelbagai ejen priming. Oleh itu, eksperimen ketiga dilakukan untuk mengkaji kesan asid humik (HA) (ejen priming yang mesra alam sekitar) ke atas aktiviti enzim antioksida, peroksidasi lipid pada biji benih kacang soya yang sudah lama. Biji benih dengan kadar percambahan 60±5% selepas 12 bulan penyimpanan digunakan dalam eksperimen ini dan priming dengan kadar larutan air suling dan 0.2g/L HA dalam suhu 25°C selama 1, 3,5 dan 7 jam. Eksperimen di makmal dan lapangan dilakukan untuk menentukan kadar yang sesuai untuk meningkat percambahan dan kesegahan biji benih. Priming biji benih kacang soya menggunakan HA selama 5 jam dapat mengurangkan kemerosotan kualiti biji benih dengan meningkatkan aktiviti enzim antioksida katalase (CAT) dan peroksidase (POD) serta mengurangkan jumlah malondialdehid dalam biji benih. Kajian menunjukkan priming pada kadar larutan 0.2g/L HA selama 5 jam merupakan kaedah yang praktikal untuk memperbaiki kesegahan anak benih menggunakan biji benih yang berkualiti rendah. Secara rumusan, biji benih kacang soya yang melepasi kualiti minimum boleh dihasilkan di kedua-dua kawasan MI dan POL dengan melakukan penanaman pada masa yang sesuai iaitu diantara Jun sehingga Ogos (P1) dan dari Januari sehingga Mac (P2) dan boleh di simpan selama 8 bulan di dalam penyimpanan yang dimodifikasi. Keseluruhan dapatan daripada kajian ini boleh digunakan untuk membantu meningkatkan ketersediaan benih kacang soya pada masa penanaman.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

P1	Planting 1
P2	Planting 2
PTFNFP	Presidential Task Force on National Food Production Program
HA	Humic acid
ALU	Aluttarama
POL	Polonnaruwa
MI	Mhailluppalama
FG%	Final germination %
GI	Germination index
GRI	Germination rate index
MGT	Mean germination time
CVG	Coefficient velocity of germination
SVI	Seedling vigour index
GLU	Glucose
SUC	Sucrose
RFOs	Raffinose family oligosaccharide
CAT	Catalase
POD	Peroxidase
MDA	Malondialdehyde
FW	Fresh weight
DW	Dry weight
ISTA	International Seed Testing Association
LSD	Least Significant Differences

RCBD	Randomised Complete Block Design
SAS	Statistical Analysis Software
CRD	Complete Randomized Design
p-value	Probability level
PM	Physiological maturity
HM	Harvesting maturity
ANOVA	Analysis of variance
df	Degree of freedom
NS	Normal/ Ambient stores
MS	Modified/ Air-conditioned store
mt	Million metric tons
SL	Seed lots
ST	Storage type
SP	Storage period
PE	Production environment
HMS	Harvesting maturity stages
YSM	Yield per square meter

CHAPTER 1

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a legume belonging to the family Leguminosae and subfamily Papilionoidea (Nguyen & Bhattacharyya, 2017), which grows well in tropical, subtropical and temperate climates. Soybean plays an important role in the world food supply and is the second-largest contributor to the world vegetable oil production after oil palm. Based on the extent of cultivation, it is ranked fourth in the world after wheat, rice and maize (Rębilas et al., 2020). Soybean seeds contain 20% oils and 40% protein (Meena, 2017), hence it is considered as a valuable source of protein.

Soybean is deemed important in Sri Lanka because of the country's growing demand for feed in the livestock industry, and in human nourishment. To date, around 235,000 t of soybean is required annually in Sri Lanka. (15,000 t for human and 220,000 t for animal feed) (Ministry of Agriculture, 2017). More than 90% of the total national requirement is imported, utilizing a large amount of money (65 million US Dollars in 2015) for soybean importation and it is expected to increase yearly (Presidential Task Force on National Food Production (PTFNFP), 2015). Currently, soybean is cultivated in two growing seasons per year, the main cultivation season (dry) being May to September (84%), while the off season (wet) is in November to February with only 16% of the total cultivation (7000 ha) for the period 2013-2017. To reduce importation, the production of local soybean must be increased by increasing productivity as well as expansion of cultivation area. There is a huge potential exists to increase the soybean cultivation in Sri Lanka specially in the dry season by utilizing partially abandoned paddy land due to water shortage (Wijesekera, 2015). Therefore, the Ministry of Agriculture's mission is to increase the extent of cultivation up to 42,000 ha from the current 7,000 ha to reduce the importation of soybean (PTFFP, 2015). The expansion plan can only succeed if it is supported by the production and supply of quality seeds at the time of planting.

Currently, seed production activities of the country are being carried out at 16 seed production regions specialized for one or a few selected crops based on the environmental suitability, farmers preferences and experience (Weerasekara et al., 2012; Kariyawasam et al., 2018). Accordingly, soybean seed production is mainly carried out at the Anuradhapura district [Mahaillupallama (MI) seed production region] and concentrates on a single variety (PB-1) due to unavailability of other recommended varieties. Based on the reports of Department of Agriculture (DOA), soybean seed production stagnated around 400-500 t per year, barely meeting the current cultivation requirement. The production centers are not equipped with seed storage facility causing more than 20% of produced seed lots to lose their viability before the next planting season. However, if land area planted with soybean is to be increased, a concomitant increase in the availability of quality seeds must triumph. Hence, the current seed production program which focuses at MI seed production region must be intensified by the introduction of new seed production areas for a four-fold increase in the amount of quality seeds required for the total planting hectarage.

Seed quality is a vital attribute for crop production and food security (Finch-Savage & Bassel, 2016). According to the Sri Lankan seed quality standard, a minimum germination level of 75% must be maintained prior to planting and it is the main challenge faced by the seed producers. Therefore, to address the quality issue of soybean it is important to evaluate the seed from pre- and post-harvest perspective. Pre-harvest encompasses the seed development phases upon successful pollination and fertilization. During development process, the seed will undergo a series of morphological and physiological changes which will eventually influence the quality of the harvested seeds (Baskin & Baskin, 2014). However, limited information is available on quality changes of soybean under different production environments. Thus, an attempt was made to investigate the effect of production environment on seed quality of soybean with the aim to recommend areas suitable for seed production.

In view of the current soybean cultivation pattern of the country, main seed demand occurs just before the beginning of the dry season (main season). To fulfill the demand, seeds produced from the previous two seasons will be used. Thus, seed storage is a prerequisite to maintain the viability of the seeds and it is considered as a critical post-harvest factor which determines the final seed quality prior to planting. In Sri Lanka, soybean seeds are stored under ambient store condition where fluctuation in temperature (25 – 34 °C) and relative humidity (37-87%) occurs. It is reported that fluctuating seed storage conditions are harmful for seed viability and longevity (Jyoti & Malik, 2013). Most of the research findings have suggested to store the seeds under ideal conditions such as low temperature (<15 °C) and low humidity (<50%) to retain viability during seed storage. However, due to the hot tropical conditions in Sri Lanka, it is difficult to achieve and to maintain the above-mentioned conditions without a huge investment, which is economically not viable for both farmers and commercial seed producers. Therefore, it is imperative to introduce economically viable system for commercial seed producer to ensure minimum losses of seed quality during storage. The installation of air conditioners in the stores will allow the maintenance of the temperature at 20 °C and humidity around 50-60%, which according to McCormack, (2010) will be the ideal solution with considerably low investment for the mid-level seed handlers. However, the effectiveness and advantage of using air-conditioning to prolong seed longevity has not been evaluated until now.

Even under ideal storage conditions, seed quality losses are unavoidable. As a result, seeds maybe be viable but have low vigour producing poor seedling quality upon field planting, delayed germination and contribute to uneven field establishments. Pre-sowing seed improvement treatments, play a critical role in instilling repair mechanisms in aged seed (Komala et al., 2018; Marthandan et al., 2020), through changes in the metabolic processes at the early stages of germination. Seed priming is a popular pre-sowing seed enhancement technique, in which seeds are treated with some aqueous solution of priming agents whereby the seed is moderately hydrated to the point where pre-germination metabolic processes are initiated without actual germination, followed by dry back to its original moisture level (Ocvirk et al., 2021). The priming process stimulates enzyme activation and protein synthesis, as well as cell membrane restoration and antioxidant defence mechanisms (Mohamed et al., 2018), and the collective result is rapid and uniform germination, improved seedling vigour, and growth of seeds via initiation of repair mechanism (Malek et al., 2019; Vieira et al., 2018). To improve the seed and seedling vigour, numerous seed priming procedures are being employed, including water (hydropriming), osmotic solutions (osmopriming), and matric materials (matricpriming). In previous investigations on priming of soybean seeds, numerous priming agents were recommended such as polyethylene glycol (PEG) (Thant et al., 2017), salicylic acid (SA) (Nazari et al., 2020), potassium nitrate (KNO_3), potassium chloride (KCl) and ascorbic acid (ASA) (Miladinov et al., 2020). The above-mentioned chemicals have shown positive effect, although, concerns of its use on the environment and the search for user-friendly seed priming agent for sustainability is on-going. Thus, environmentally friendly additives for seed priming are becoming a trend. Humic acid (HA) is considered as an environmentally friendly seed additive, since it is the primary decomposition product of plant and animal tissues and showed positive results in various crops such as chilli (Ananthi et al., 2017), sesame (Souguiri & Hannachi, 2017), wheat (Nazi et al., 2014; Patil et al., 2010) and pea (Gawlik et al., 2013). According to the findings of these investigations, the efficacy of HA priming is dependent on the concentration and soaking time, which differ for each crop. To date, there has been no report on the use of HA as a soybean priming agent, specifically on the repair processes based on enzyme activation and reduction of peroxidation activity.

Based on the background information provided above, this study was conducted with the following objectives :

1. To study the effect of different production environments on seed development, harvest maturity and quality of soybean seeds, cultivar PB-I.
2. To determine the longevity of soybean seeds, cultivar PB-1, produced in different production environments upon storage under ambient and air-conditioned room.
3. To investigate the use of humic acid (HA) as a potential priming agent for invigoration of stored soybean seeds.

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