JOURNAL OF AGRICULTURE AND APPLIED BIOLOGY
2023, Vol. 4, No. 2, 143 - 150
http://dx.doi.org/10.11594/jaab.04.02.04
E-ISSN: 2723-5106

Research Article

Application of ι-carragenan/agarose hydrogel as super adsorbent hydrophilic polymers natural seed coating for improving tobacco seed germination under drought stress

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Article history:

Submitted 4 July 2023
Accepted 23 August 2023
Published 22 September 2023

Keywords:

Abiotic stress
Organic coating
Seed technology
Tobacco
Sustainable farming

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Abstract

Seed storage for a long time causes seed deterioration. Reduction of germination rate can cause abnormal growth of the seedling, which impacts the yield and quality of tobacco plants. Seed coating is an additive that coats the seeds with certain ingredients such as pesticides, and nutrients that are glued to a binding material for improved seed performance without changing its origin's form. Hydrogel seed coating is also natural and environmentally friendly which is suitable for organic and sustainable farming practices. In countries with worse droughts and depending on natural plants, natural seed coating using agarose and Carragenan is very well applied. Carrageenans regulate many of the metabolic pathways involved in nitrogen and sulfur absorption, making it easier to grow plants by regulating a variety of metabolism processes including photosynthesis as well as an ancillary pathway. In conditions of drought stress, salicylic acid treatment may have a beneficial effect on plant resistance. In plants, salicylic acid regulates the growth of endogenous cells and plays an important role in abiotic stress signal transduction. The present field experiment was conducted to evaluate the effectiveness of ι-Carragenan/Agarose hydrogel as super adsorbent hydrophilic polymers natural seed coating with 3 type combination of ι-Carragenan and Agarose (1:1; 1:2 and 2:1)% w/w containing salicylic acid 0.15% w/w to improve sprouting speed index, sprouting percentage, fresh seed mass and seed vigor index of tobacco seeds which are 3 variety storage time (13 years, 9 years and 3 years) under drought stress condition (40% moisture levels). The results revealed that application of ratio ι-Carragenan/Agarose 1:2 and 2: 1 % w/w containing salicylic acid 0.15% w/w recommended of Super Adsorbent Hydrophilic polymers Natural Seed Coating formulation enhanced of sprouting speed index, germination percentage, and seed vigor index of 2 variety storage time of tobacco seed (13 years and 9 years).

How to cite:

Arum, A. P. & Setiyono (2023). Application of ι-carragenan/agarose hydrogel as super adsorbent hydrophilic polymers natural seed coating for improving tobacco seed germination under drought stress. *Journal of Agriculture and Applied Biology, 4*(2): 142 - 150. doi: 10.11594/jaab.04.02.04

1. Introduction

Seed storage is an activity carried out to maintain seed viability in long-term period (Indriana, 2016). Long time seed storage can cause seed deterioration. Seed deterioration is caused of seed genetic and environmental factor. Seed deterioration due to genetic factor is known as the chronological deterioration process. While seed deterioration occurs resulting in loss of crop quality, viability, and vigor due to environmental factors. Seed aging during storage may cause retardation of field establishment, and may eventually result in seedling abnormalities or even failure of emergence (Shaban, 2013). Moreover, seed deterioration during storage is affected by genetic background and motherhood environmental (Nagel et al., 2015). Seed coating is coating treatment of seeds with some ingredients like pesticides, nutrients which is glued by the binding material for improving seed performance without changing the origin form of seeds (Javed et al., 2022).

The result of many research showed that seed coating on wheat seed (*Triticum durum Desf.*) is approved using of mixture Agarose and ι-Carragenan as natural hydrogel seed coating on wheat seed can show better growth performance than wheat seed without coating under drought stress conditions (Hotta et al., 2016). Hydrogel seed coating is also natural and environmentally friendly which is suitable for organic and sustainable farming practices. Comparation of natural seed coating using agarose and ι-Carragenan and synthetic seed coating using Polyacrylamide (PA) on wheat seed show natural seed coating better germination growth than synthetic seed coating during ideal growth (80% humidity levels) and aridity stress situation (40% moisture levels) (Hotta et al., 2016). Natural seed coating using agarose and ι-Carragenan is very well applied to countries that have worse drought conditions and depending on natural plants.

One of technique for increasing resistance in drought stress of plant is salicylic acid treatment. The research from (Arif et al., 2020; Miura & Tada, 2014; Sedaghat et al., 2017; Tayyab et al., 2020; Hosain et al., 2020) show salicylic acid treatment can increase the resistance of corn, wheat and rice in drought stress conditions. Salicylic acid is a regulator of the growth of endogenous plants that play an important role in the transduction of abiotic stress signals in plants (Arif et al., 2020). Furthermore, Tobacco seeds is coated with of poly acid (2-acrylamide-2-methyl propane sulfonic acid) (PAMPS) hydrogel seed coating containing of salicylic acid (0.15 g / 100 ml) can improve the germination and growth performance of tobacco seeds during drought stress conditions (Guan et al., 2014). Likewise, wheat seed which is soaked with salicylic acid at low concentrations can accelerate the germination time and increase the percentage of wheat germination in drought stress conditions (Sharafizad et al., 2013). Therefore, the current study was planned to evaluate the effectiveness of ι-Carragenan/Agarose Hydrogel as Super Adsorbent Hydrophilic polymers Natural Seed Coating with with 3 type combination of ι-Carragenan and Agarose (1:1; 1:2 and 2: 1) % w/w containing salicylic acid 0.15% w/w to improve sprouting speed index, sprouting percentage, fresh seed mass and seed vigour index of tobacco seed with 3 variety storage time of tobacco seed (13 years, 9 years and 3 years) under drought stress condition (40% moisture levels).

2. Materials and methods

2.1 Experimental design and treatments

The effect of of ι-Carragenan/Agarose Hydrogel as Super Adsorbent Hydrophilic polymers Natural Seed Coating with 3 type combination of ι-Carragenan and Agarose (1:1; 1:2 and 2: 1) % w/w containing salicylic acid 0.15% w/w to improve sprouting speed index, sprouting percentage, fresh seed mass and seed vigour index of tobacco seed with 3 variety storage time of tobacco seed (13 years, 9 years and 3 years) was examined at two distinct moisture levels in a lab setting (80% for ideal growth conditions and 40% for simulation of drought conditions). Materials was used in this research are tobacco seed (Nicotiana tabacum) variety of H382 from PTPN X tobacco plant research and development center, iota-carragenan (Sigma Aldrich), Agarosa (Sigma Aldrich), Asam Salisilat (Sigma Aldrich), Talk (Sigma Aldrich) dan Bentonite (Sigma Aldrich) for seed coating. The seeds were coated using a conventional coating machine with spraying system.

24 plastic containers with a pleated sheet inside that have had the moisture level regulated to 80% for simulation normal growth were employed to determine twelve handling in two factor (3 variety storage time of H382 tobacco seed that is 13 years, 9 years and 3 years and four seed coating formulation that is 3 type combination of ι-Carragenan and Agarose (1:1; 1:2 and 2: 1) % w/w containing salicylic acid 0.15% w/w and without seed coating, randomised completed design with two replicates. the treatment is the same as 24 other plastic containers with a pleated sheet inside (with the moisture level set to 40% for simulation drought conditions) (Tabel 1). Each plastic box containing 50 seeds had been prepared and placed in a seed chamber to keep the moisture. Observation and measurement of parameters were carried out starting 2 days until of 14 days after sowing. Seedling Measurements are improve sprouting speed index (GSI), sprouting percentage (G), fresh seed mass (SW) and seed vigor index (SVI) (da Silva et al., 2017; Pereira et al., 2021):

$$GSI = \sum \frac{\text{Gn}}{\text{Dn}} \quad (1)$$

$$G\ (\%) = \left(\frac{Ga}{Nn}\right) X100 \quad (2)$$

$$SW\ (g/g) = \left(\frac{Wf}{Wi}\right) \quad (3)$$

$$\text{SVI}\ (\%) = \frac{\sum normal\ sprouts\ on\ the\ first\ count}{\sum seed\ is\ planted} X100 \quad (4)$$

Where :
Gn = The total number of seeds that have germination each day
Dn = Days following incubation, in days
Ga = The number of typical seeds that germinated on the seventh day after incubation
Nn = The total amount of experimental seeds on a single paper towel
Wf = The average mass of the seedlings
Wi = Prior to the germination test, the dry seed weight on average

Table 1. Treatment of Tobacco Seed Covering

Model Code	Coating formulation			Storage Time (years)	Moisture Level (%)
	ι-Carragenan % (w/w)	Agarose % (w/w)	Salicylic Acid %(w/w)		
F0S1M1	0	0	0	13 years	80%
F0S2M1	0	0	0	9 years	80%
F0S3M1	0	0	0	3 years	80%
F1S1M1	1	1	0.15	13 years	80%
F1S2M1	1	1	0.15	9 years	80%
F1S3M1	1	1	0.15	3 years	80%
F2S1M1	1	2	0.15	13 years	80%
F2S2M1	1	2	0.15	9 years	80%
F2S3M1	1	2	0.15	3 years	80%
F3S1M1	2	1	0.15	13 years	80%
F3S2M1	2	1	0.15	9 years	80%
F3S3M1	2	1	0.15	3 years	80%
F0S1M2	0	0	0	13 years	40%
F0S2M2	0	0	0	9 years	40%
F0S3M2	0	0	0	3 years	40%

Model Code	Coating formulation			Storage Time (years)	Moisture Level (%)
	ι-Carragenan % (w/w)	Agarose % (w/w)	Salicylic Acid %(w/w)		
F1S1M2	1	1	0.15	13 years	40%
F1S2M2	1	1	0.15	9 years	40%
F1S3M2	1	1	0.15	3 years	40%
F2S1M2	1	2	0.15	13 years	40%
F2S2M2	1	2	0.15	9 years	40%
F2S3M2	1	2	0.15	3 years	40%
F3S1M2	2	1	0.15	13 years	40%
F3S2M2	2	1	0.15	9 years	40%
F3S3M2	2	1	0.15	3 years	40%

2.2 Statistical evaluation

A one-way Analysis of Variance (ANOVA) followed by post-hoc Duncan's test was conducted to ascertain the impact of ι-Carragenan/Agarose Hydrogel as Super Adsorbent Hydrophilic polymers Natural Seed Coating with 3 type combination of ι-Carragenan and Agarose (1:1; 1:2 and 2:1) % w/w containing salicylic acid 0.15% w/w to improve sprouting speed index (GSI), sprouting percentage (G), fresh seed mass (SW) and seed vigor index (SVI) of tobacco seeds which are 3 variety storage time (13 years, 9 years and 3 years) under normal condition (80% humidity levels) and aridity stress condition (40% humidity levels). The P-value threshold for statistical significance was set at 0.05. The statistical program IBM SPSS Statistics 22 (IBM Inc, Jakarta, Indonesia) was used for every analysis.

3. Results and discussion

There was any significant effect on GSI, G and SVI among the various levels of ι-Carragenan/Agarose coverings and control seeds ($p>0.05$) at 80% humidity levels (Table 2) and 40% moisture levels (Table 3) but no significant effect on SW. Growth Speed Index (GSI), Sprouting Percentage (G) and Seed Vigor Index of coated tobacco seeds with levels of ι-Carragenan/Agarose coatings F1S1M1 and F2S1M1 were same effect with control seeds on normal condition (80% moisture levels) (Table 2). Likewise, coated tobacco seeds with levels of ι-Carragenan/Agarose coatings F2S1M2 and F3S2M2 drought stress condition (40% moisture levels) were same effect too with control seed (Table 3)

Table 2. Carragenan/Agarose coating concentration affects seed development at 80% moisture (normal conditions)

Treatment	GSI	G (%)	SVI (%)
F0S1M1	6.65^{a}	86.5^{a}	86.5^{a}
F0S2M1	4.27^{abcd}	55.5^{abc}	55.5^{abc}
F0S3M1	5.04^{abc}	65.5^{ab}	65.5^{ab}
F1S1M1	6.71^{a}	83.5^{ab}	83.5^{ab}
F1S2M1	5.38^{abc}	69.5^{ab}	69.5^{ab}
F1S3M1	0.69^{f}	9.0^{e}	9.0^{e}
F2S1M1	6.19^{a}	80.5^{ab}	80.5^{ab}
F2S2M1	5.00^{abc}	65.0^{ab}	65.0^{ab}
F2S3M1	2.31^{bcdef}	29.5^{cde}	29.5^{cde}
F3S1M1	3.88^{abcdef}	50.5^{bcd}	50.5^{bcd}
F3S2M1	5.53^{ab}	72.0^{ab}	72.0^{ab}
F3S3M1	1.53^{def}	20.0^{de}	20.0^{de}

Values at the same column followed with same letter are not significantly different (P=0.05) according to Duncan's Multiple Range Test

1. GSI is germination speed index
2. G is germination percentage
3. SVI is seed vigour index

Table 3. The influence of different -Carragenan/Agarose coating concentrations on seed development at 40% moisture (drought stress conditions)

Treatment	GSI	G	SVI
F0S1M2	5.03^{abc}	66.0^{ab}	66.0^{ab}
F0S2M2	4.30^{abcd}	56.0^{abc}	56.0^{abc}
F0S3M2	5.07^{abc}	65.5^{ab}	65.5^{ab}
F1S1M2	4.92^{abc}	64.0^{ab}	64.0^{ab}
F1S2M2	3.92^{abcdef}	51.0^{abcd}	51.0^{abcd}
F1S3M2	0.88^{ef}	11.5^{e}	11.5^{e}
F2S1M2	5.85^{a}	76.0^{ab}	76.0^{ab}
F2S2M2	3.69^{abcdef}	48.0^{bcd}	48.0^{bcd}
F2S3M2	4.07^{abcde}	14.0^{e}	14.0^{e}
F3S1M2	4.69^{abcd}	61.0^{abc}	61.0^{abc}
F3S2M2	5.84^{a}	76.0^{ab}	76.0^{ab}
F3S3M2	2.07^{cdef}	27.0^{cde}	27.0^{cde}

Values at the same column followed with same letter are not significantly different (P=0.05) according to Duncan's Multiple Range Test

1. GSI is germination speed index
2. G is germination percentage
3. SVI is seed vigour index

From this study, it was found that treatment of ι-Carragenan/Agarose coatings concentration (1:1) %w/w containing salicylic acid 0.15% (w/w) on tobacco seeds 13 years storage time showed the highest GSI (6.71), G (83,5%) and SVI (83,5%) than other treatments under 80% moisture levels (normal conditions). (Fig.1). Treatment of ι-Carragenan/Agarose coatings concentration (1:2) %w/w and (2:1)% w/w containing salicylic acid 0.15% w/w on tobacco seeds 13 years and 5 years storage time showed the higher GSI (5.85 and 5.84), G (76% and 76%) and SVI (76% and 76%) than other treatments under 40% moisture levels (drought stress conditions). (Fig.1). Seed coating using ι-Carragenan/Agarose containing salicylic acid 0.15% (w/v) can retain good germination growth under drought stress conditions (40% moistures level). ι-Carragenan/Agarose (2:1)% w/v seed coatings gave the best results of germination index 32% and 36% than without seed coating 31% and 34% on Durum wheat and Bread wheat seeds (Hotta et al. 2016). In addition, ι-Carragenan/Agarose (2/1)% w/v will provide a layer that is not too hard to be penetrated by radicles and plums that are very soft from seeds (Kow et al., 2015).

Using of ι-Carragenan as a seed coating material that ι-Carragenan consists of carbohydrates such as sugar and other mineral Mg, K, Ca, Na and S. By providing additional nutrients, these chemical components can influence seed growth, or the impact of biological processes such as enzyme activity on root and stem development (Ali et al., 2021; Deolu-Ajayi et al., 2022; Prajapati et al., 2014). Other component, that was used as seed coating in this study was salicylic acid (SA). Salicylic acid (SA) is widely considered to be an endogenous plant growth regulator that plays an important role in the signal transduction pathway of abiotic stresses in plants (Arif et al., 2020). Increasing Salicylic acid exogenous is able to increase resistance to drought stress that has been observed in many plants such as corn (Tayyab et al., 2020), wheat (Miura & Tada, 2014; Sedaghat et al., 2017), and rice (Hosain et al., 2020). The drought tolerance mechanism of the wheat crop

which is exposed to drought stress has been enhanced by application of salicylic acid and its derivatives for use infoliant or seed treatment applications. Research has shown that when salicylic acid is applied to wheat, increases in the level of abscisic acid have an indirect effect on proline accumulation (Miura & Tada, 2014; Sedaghat et al., 2017). The high content of ι-Carragenan makes easy for radicles and plums to penetrate the coating layer because because the polysaccharide -Carragenan employed in this investigation is high in sulfate content compared to agarose (Rocha et al., 2014) and the structure is soft and elastic gels, whereas agarose forms stiffer structures and less elastic gels. (Ghebremedhin et al., 2021).

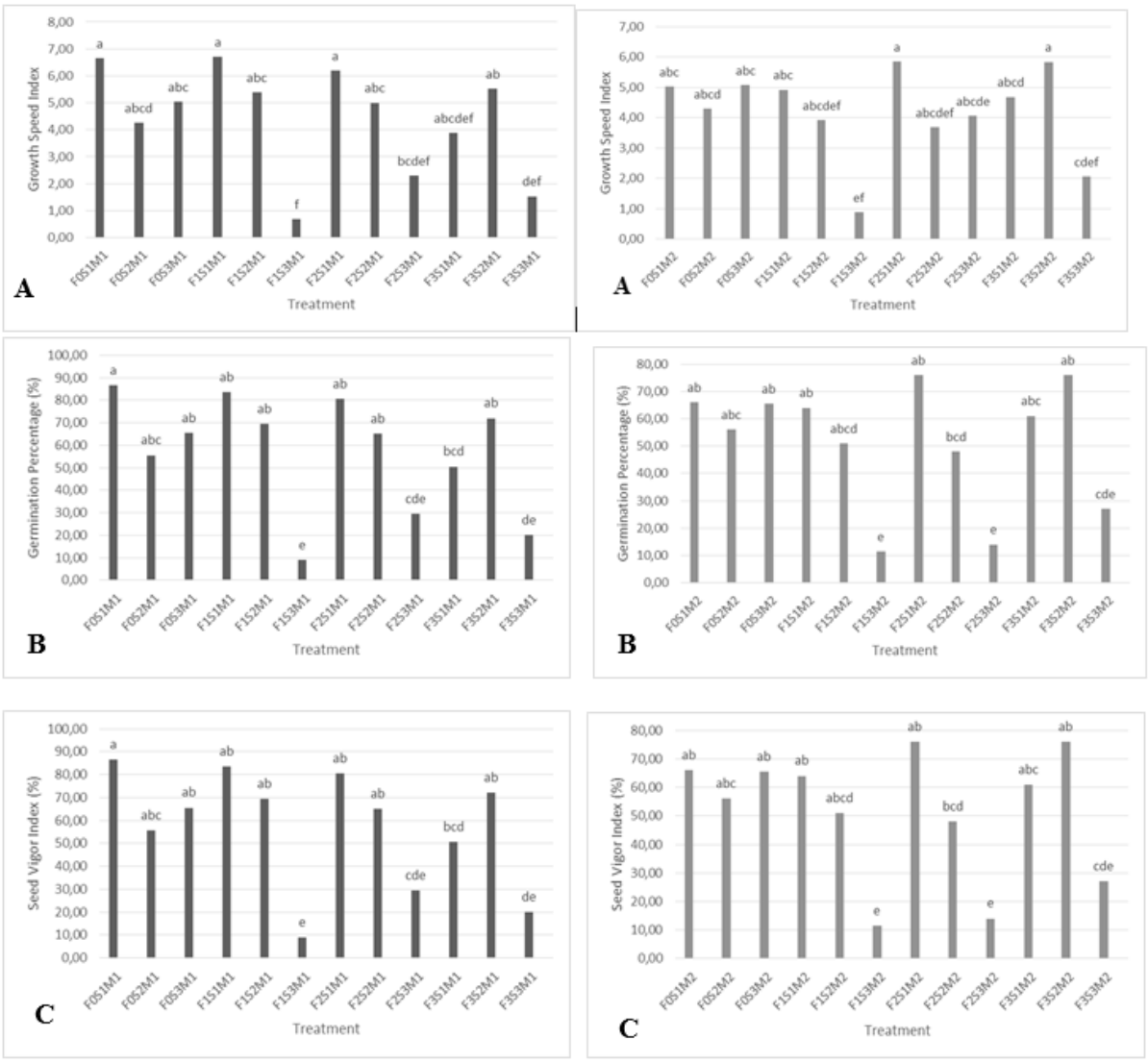

Figure 1. Germination speed index (A), germination percentage (B) and seed vigor index (C) of tobacco seeds 13 years, 9 years and 3 years storage time coated with 1/1;1/2 and 2/1 % w/v ι-Carragenan/Agarose (dark grey bars) tested in 80% moisture level (normal conditions) and (light grey bars) tested in 40% moisture level (drought stress conditions)

4. Conclusion

Seed coating using ι-Carragenan/Agarose containing salicylic acid 0.15% (w/v) on tobacco seeds showed good influence on seed growth under normal situations (80% humidity level) and

drought conditions (40% humidity level). The seedling performance improved significantly on the sprouting speed index (GSI), sprouting percentage (G), and seed vigor index (SVI) in comparison to seeds without coating. But, the seed coating did not effect SW (fresh weight seed). In conclusion, ι-Carragenan/Agarose containing salicylic acid showed an advantage as natural seed coating regarding of seedling growth under dry stress. The use of a seed coating technique that increases drought resistance may be a new method to improve the establishment of seeds in stressful conditions. Nevertheless, to ensure that the plant is able to cope with stress, an assessment of this method should be carried out in additional seed crops and adaptations made using a variety of natural ingredients must be introduced.

Acknowledgement

This research was supported by the Department of Research and Community Service University of Jember Fund 2018. We would like to thank Balai Penelitian dan Pengembangan Tembakau PT. Perkebunan Nusantara X for the supply of tobacco seeds.

Author's declaration and contribution

The authors certify that they have no conflicts of interest. AP: carried out all experiments and writing of research result, SS (Lecturer of Agronomy) was supervised in this study.

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