American Journal of Agricultural and Biological Sciences

Original Research Paper

Durum Wheat Seed Germination Response to Hydrogel Coatings and Moisture under Drought Stress

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Article history
Received: 15-10-2015
Revised: 24-03-2016
Accepted: 27-04-2016

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Abstract: Technologies that can increase crop production while maintaining low pollution are essential for sustainable agriculture. This study compares the germination performance of Durum wheat seeds (*Triticum durum* Desf.) which were coated with synthetic coatings [polyacrylamide (PA)] and novel natural coatings [mixtures of agar/ι-carrageenan (AC)] at different moisture levels. A number of germination performance indicators were measured for each of the categories of coatings. These included: (i) % germination, (ii) radicle emergence, (iii) speed of germination, (iv) seedling length, (v) fresh seed weight and (vi) seed vigour index. Results showed that both the synthetic and natural coatings improved germination performance in the Durum wheat seeds versus the untreated seeds when non-drought (80% moisture) conditions were used. However, when 40% moisture levels were used, the natural coated seeds showed a 6% improvement in germination performance over the synthetic PA coated seeds. This improved germination performance under drought stress along with the fact that these novel AC coatings used are completely natural and environmentally friendly suggests that these novel coatings have a big future in seed coatings, especially in countries which experience drought conditions and are dependent on natural irrigation.

Keywords: Drought Test, *Triticum Durum* Wheat, ι-Carrageenan, Polyacrylamide, Seed Coating

Introduction

There is a demand to use environmentally friendly seed coatings made from natural polymers due to contamination of soils and the surrounding environment with toxic and persistent materials (Zeng and Wang, 2010; Zeng and Zhang, 2010). With climate change and the unpredictability of rainfall, the development of green technologies that can provide both environmental protection and drought resistance in crops has become a matter of great importance (Guan *et al*., 2014; Kneipp, 2008).

This project uses water-absorbent polymers as seed coating materials in order to support sustainable agriculture and provide solutions to the food crisis. The work investigates the difference in seed germination and seed vigour of Durum wheat seeds coated with a synthetic linear Polyacrylamide (PA) polymer versus a novel Agar/ι-Carrageenan (AC) blended natural hydrogel. Tests were undertaken using ideal growth conditions (80% moisture levels) and simulated drought conditions (40% moisture levels) in the laboratory.

Recent weather problems, particularly drought, have resulted in a decrease in agricultural production yield worldwide (FAO, 2016; NASA, 2015; ZernoExport, 2013). In some countries wheat production has reduced in situations where drought has been severe (Kneipp, 2008; Nijhuis, 2014; Taylor and Koo, 2012). Reduced seed germination due to these drought conditions has contributed significantly lower wheat yields. Seed suppliers have attempted to address this problem by producing a new class of seed coating called Super-Absorbent hydrophilic Polymers (SAPs) which include PA polymers. The mechanism of action of these polymer coatings to aid the seed germination process depends on their chemical composition and how they are applied on the seeds (Ekebafe *et al*., 2011; Landis and Haase, 2012; Puoci *et al*., 2008; Rudzinski *et al*., 2002; Yang *et al*., 2014; Zohuriaan-Mehr and Kabiri, 2008).

Some seed coatings based on SAPs can help improve percentage germination, speed up germination time, promote root/shoot development in the early stages of plant growth and accelerate the harvest time (Akelah, 2013). SAPs used in agriculture are categorised into synthetic, modified-natural and natural polymers. Polyacrylamide (PA) is a synthetic polymer that can be synthesised into either a linear or a gelling system (Fig. 1). Linear PAs are used for erosion control, canal sealing and water clarification, whereas gel-forming cross linked PAs are used in horticulture and are commonly referred to as hydrogels (Landis and Haase, 2012). Synthetic polymers have the benefit of being mechanically strong and long-lasting compared to natural polymers. PA itself does not pose any environmental threat (Seybold, 1994) and is subject to thermal and photo-degradation processes as well as mechanical breakage by soil cultivation. However, the residual acrylamide (AAm) content in PA is a concern. The monomer is a potentially carcinogenic material and a known neurotoxin to humans and animals (Sojka *et al*., 2007). The U.S. Food and Drug Administration (FDA) regulates the amount of residual AAm to be less than 0.05% in PA resin for food treatment for human consumption (FDA, 2015; Seybold, 1994; Sojka *et al*., 2007). Nevertheless acrylamide does not accumulate in soil and it is hydrolysed into less harmful chemicals (Seybold, 1994). Purification of polyacrylamides to remove residual monomers and increase its stability is a main concern (Caulfield *et al*., 2003) dealing with which increases manufacturing costs substantially.

Natural polymers are preferred to synthetic polymers as they are inert, safe, non-toxic, biocompatible, biodegradable, low costing, eco-friendly and abundantly available in nature (Prajapati *et al*., 2014). However, their chemical composition tends to be complex and varied from batch-to-batch. They are also mechanically weaker and more susceptible to degradation than synthetic polymers (Martin *et al*., 2011; Puoci *et al*., 2008). Carrageenans and agar are natural polymers derived from red seaweeds widely used in the food, cosmetic and pharmaceutical industries (Imeson, 2000; Meena *et al*., 2009; Pereira *et al*., 2003; Prajapati *et al*., 2014). These polysaccharides have the ability to form hydrogels when dissolved in hot water (over 85°C) and left to cool. The strength and gel forming ability of these galactose-containing polysaccharides depends on the level of sulphate ester ($O\text{-}SO_3^-$) groups present in the structure. The ι-carrageenan used in this study is a highly sulphated polysaccharide compared to agar (Cregut and Rondags, 2013; Cyber Colloids Ltd, n.d.; Imeson, 2000; Rocha *et al*., 2014; Smith and Hong-Shum, 2003) and this structure gives a relatively soft and elastic gel, while agar tends to form a stronger and less elastic gel (Lyons *et al*., 2009) (Fig. 2).

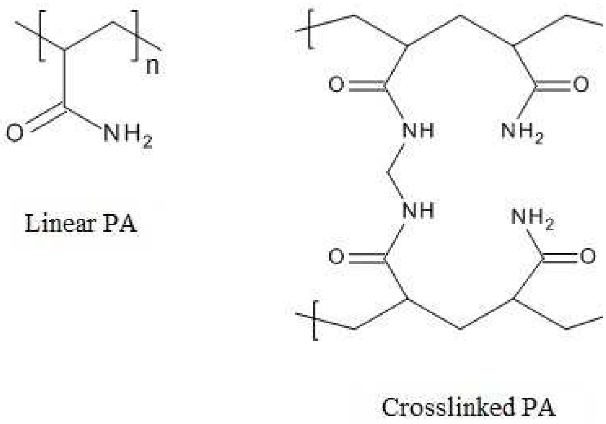

Fig. 1. The chemical structure of linear polyacrylamide (left) and gel-forming polyacrylamide (right)

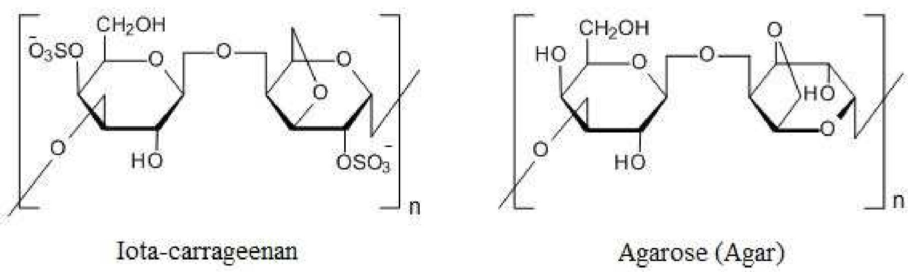

Fig. 2. The chemical structure of ι-carrageenan (left) and agar (right)

Materials and Methods

The effect of two coating agents (Table 1) including polyacrylamide (PA) and agar/ι-carrageenan blend (AC) on Durum wheat seeds germination was investigated under laboratory conditions in two different moisture levels. All samples were pre-chilled for one day at 6°C and then transferred into an incubator maintained at a constant temperature of 20±2°C for a total of 7 days, having a day/night cycle of 8/16 h.

The seeds were coated using a Satec Concept ML 200 seed coating machine.

Seed Germination Tests under High Moisture Level

The experiment was carried out in 80% moisture to provide ideal growth conditions for Durum wheat seeds following the International Seed Testing Association guidelines (ISTA, 2015). The purpose of this study was to investigate the effect of polymer coating volumes and polymer coating types on the germination of seeds.

Ten plastic boxes containing a pleated sheet (with moisture level adjusted to 80%) were used to establish ten treatments in a two-factor, randomised block design with four replicates. Hundred seeds were sown in each sheet. The treatments included 5 levels of coating volumes; 0, 2, 4, 8 and 16 mL and two types of polymer coating formulations; PA and AC (Table 2).

Seed Germination Tests under Low Moisture Level

The experiment was carried out in 40% moisture to simulate drought condition on Durum wheat seeds. The purpose of this study was to compare the germination response of Durum wheat seeds between untreated seeds (control) and polymer coated seeds under less than ideal growth conditions. A randomised block design with a single factor consisting of 3 treatments; Control, 16 mL of PA treated seeds (PA16) and 16 mL of AC treated seeds (AC16) were employed in this study (Table 2). For each treatment type, eight rolled paper towels (adjusted to 40% moisture) containing 25 seeds were prepared and placed in a sealed container to retain the moisture. This test was run in triplicate.

Seedling Measurements

The Radicle emergence (*RE*), Germination Speed Index (*GSI*), Germination percentage (*G*), fresh Seed Weight (*SW*), Seedling Length (*SL*) and Seed Vigour Index (*SVI*) were measured for each of the categories of coating as seed growth indices.

GSI, *G*, *SW* and *SVI* were calculated by using Equation 1-4 (Bonfim *et al*., 2010; Kharkale *et al*., 2011):

$$GSI = \sum \frac{Gn}{Dn} \tag{1}$$

$$G(\%) = \left(\frac{Ga}{Nn}\right) \times 100 \tag{2}$$

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

$$SVI = \left[\left(plumule + root\ length\right) mm\right] \times G(\%) \tag{4}$$

Where:

- Gn = The total number of germinated seeds observed daily
- Dn = The number of days after incubation
- Ga = The number of normal germinating seeds recorded on the 7th day after incubation
- Nn = The total number of experimental seeds per paper towel
- Wf = The average weight of the seedlings
- Wi = The average weight of the dry seeds before the germination test

Table 1. Composition of natural and synthetic seed coating solutions

Sample code	ι-Carrageenan (% w/v)*	Agar (% w/v)	Polyacrylamide (% w/v)
PA	-	-	1.5
AC	1	0.5	-

*The % concentrations are based on solids dissolved in distilled water (g/100 mL)

Table 2. Coating types and polymer application rates used for wheat seed coatings

Sample code	Coating type	Coating volume* (mL)
Control	none	0
PA2	(1.5% w/v) polyacrylamide	2
PA4	(1.5% w/v) polyacrylamide	4
PA8	(1.5% w/v) polyacrylamide	8
PA16	(1.5% w/v) polyacrylamide	16
AC2	(1.5% w/v) agar/ι-carrageenan	2
AC4	(1.5% w/v) agar/ι-carrageenan	4
AC8	(1.5% w/v) agar/ι-carrageenan	8
AC16	(1.5% w/v) agar/ι-carrageenan	16

*The coating application rates are based on seed weight per volume (48 g mL^{-1})

The *RE* was measured on the 3rd day of incubation (20±2°C). The *GSI* was measured by counting the numbers of emerged seedlings on a daily basis starting from the 3rd day of incubation until the end of the test period. Seeds were considered to have germinated when the radicle was over 2 mm long (Lamhamdi *et al*., 2011). The *G*, *SL*, *SW* and the *SVI* were measured on the 7th day. The seedling length was measured for 30 seedlings from each treatment. Seed lots producing taller seedlings and higher *SVI* were considered more vigorous than the seed lots producing shorter seedlings and lower *SVI* (Gupta, 1993).

Statistical Analysis

A two-way Analysis of Variance (ANOVA) followed by post-hoc Tukey's test was used to determine the effect of factor one (Coating type) and factor two (Coating volume) on seeds. Factor one has 2 levels of coating type; polyacrylamide and agar/ι-carrageenan blend. Factor two has 5 levels of coating volume; 0, 2, 4, 8 and 16 mL. In 40% moisture conditions, one-way ANOVA with Tukey's test was used to compare the seed response to the coating material. All the estimations were analysed and the values expressed as mean ± standard error. Statistical significance was defined as *P*-values of 0.05. All analysis was conducted using the statistical package Minitab® 16 (Minitab Inc, State College, United States).

Results

Comparison of Growth Response between the Control and Polyacrylamide Coated Seeds

There was no significant difference in *G* and *SL* between the different levels of PA coatings and the control seeds ($p>0.05$) at 80% moisture level. However, a significant difference was observed in the *RE* ($F_{4, 15} = 7.15$, $P = 0.002$), the *GSI* ($F_{4, 15} = 5.42$, $P = 0.007$) and the *SVI* ($F_{4, 95} = 2.81$, $P = 0.03$) between the PA2 coated seeds and the control seeds. In the *RE* measurement, the germination rate of the control seeds were significantly lower than all the other coated seeds and for the *GSI*, the coated seeds PA2, PA4 and PA16 were significantly higher when compared to the control seeds. The *SVI* values were shown to display a significant difference between the PA2 and PA16 treated seeds, suggesting that higher PA volumes give better growth response for the Durum wheat seeds (Table 3).

Comparison of Growth Response between Control and Agar/ι-Carrageenan Coated Seeds

There was no statistically significant difference in any of the collected data among the control and the AC coated seeds ($p>0.05$). The mean values given in Table 4 shows that AC8 and AC16 samples have the highest means in terms of *SL* and *SVI*, while the highest means for *RE*, *GSI* and *G* tended to concentrate more on AC2 samples, but none were statistically significant values.

Comparison of Growth Response between Different Levels of Polyacrylamide and Agar/ι-Carrageenan Coated Seeds

The effect of seed coating type and various seed coating volume on the performance of seed growth was investigated based on the following parameters; *RE*, *SL*, *G*, *GSI* and *SVI*.

The *RE* was significantly affected by the coating types ($F_{1, 24} = 6.16$, $P = 0.02$; Fig. 3A). Coating AC gave a significantly higher germination rate when compared to the PA coated seeds. However, there was insufficient evidence to show that the difference in coating volume affected the *RE* of the seeds.

Table 3. Effect of varying polyacrylamide (PA) coating concentrations on seed growth at 80% moisture

Sample code	*RE* (%)	*GSI*	*G* (%)	*SL* (cm)	*SVI*
Control	75^{a*}	29.0^{a}	91^{a}	17.03^{a}	1550^{ab}
PA2	86^{b}	30.8^{b}	92^{a}	16.62^{a}	1533^{b}
PA4	87^{b}	30.9^{b}	93^{a}	17.51^{a}	1619^{ab}
PA8	85^{b}	30.2^{ab}	92^{a}	16.92^{a}	1553^{ab}
PA16	89^{b}	31.0^{b}	95^{a}	17.74^{a}	1677^{a}

Radicle Emergence (*RE*): Germination Speed Index (*GSI*); Germination percentage (*G*); Seedling Length (*SL*); Seed Vigour Index (*SVI*).
*Means that do not share a letter are significantly different ($p<0.05$) according to the Tukey's test

Table 4. Effect of varying Agar/ι-Carrageenan (AC) coating concentrations on seed growth at 80% moisture

Sample code	*RE* (%)	*GSI*	*G* (%)	*SL* (cm)	*SVI*
Control	79^{a*}	29.7^{a}	93^{a}	17.37^{a}	1607^{a}
AC2	90^{a}	31.3^{a}	93^{a}	17.20^{a}	1604^{a}
AC4	90^{a}	31.3^{a}	92^{a}	17.70^{a}	1619^{a}
AC8	89^{a}	31.1^{a}	90^{a}	18.52^{a}	1671^{a}
AC16	90^{a}	30.8^{a}	90^{a}	18.51^{a}	1671^{a}

Radicle Emergence (*RE*); Germination Speed Index (*GSI*); Germination percentage (*G*); Seedling Length (*SL*); Seed Vigour Index (*SVI*).
*Means that do not share a letter are significantly different ($p<0.05$) according to the Tukey's test

A significant effect on the *SL* was found in both the coating type and the coating volume. The AC coated seeds showed significantly higher *SL* than PA coated seeds ($F_{1, 152} = 7.27$, $P = 0.008$; Fig. 3B) and in terms of coating volume the 2 mL coated seeds were significantly shorter ($F_{3, 152} = 3.04$, $P = 0.031$; Fig. 3B) than 16 mL coated seeds. Nevertheless there was no interactive effect on the seeds, suggesting that the type and volume acted independently on *SL*.

On the other hand, the *G* and *GSI* showed no significant difference based on coating type, coating volume or interaction ($p>0.05$; Fig. 3C and 3D). No significant effect in coating type and interaction was found in *SVI* as well (Fig. 3E). However, it was still suspected that there may be a potential difference in seed vigour caused by the change in coating volume ($F_{3, 152} = 2.61$, $P = 0.053$), since the *P*-value was close to 0.05.

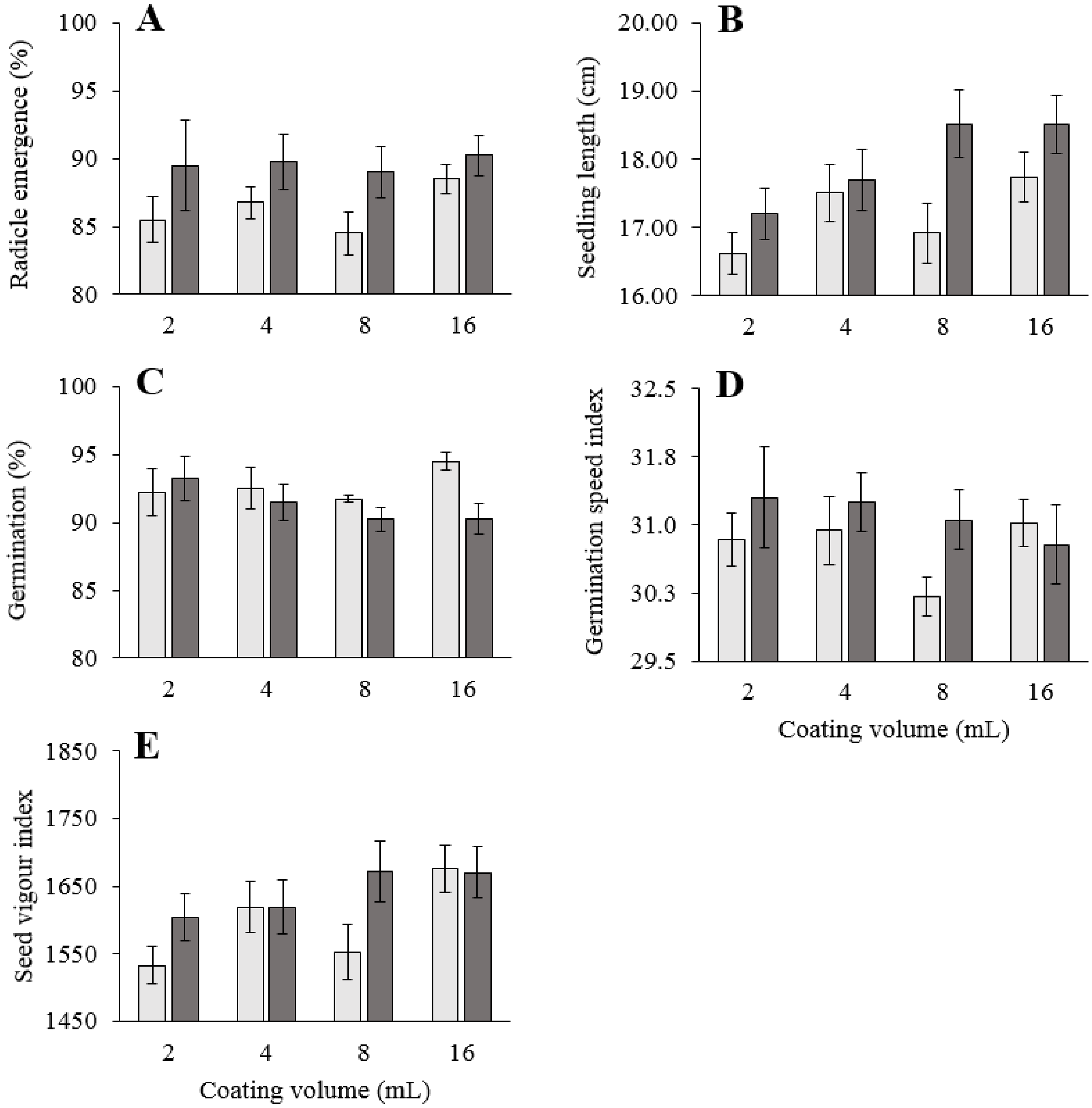

Fig. 3. The radicle emergence (A), seedling length (B), germination percentage (C), germination speed index (D) and seed vigour index (E) of Durum wheat seeds coated with 2, 4, 8 and 16 mL of polyacrylamide (light grey bars) and agar/ι-carrageenan (dark grey bars) tested in 80% moisture level

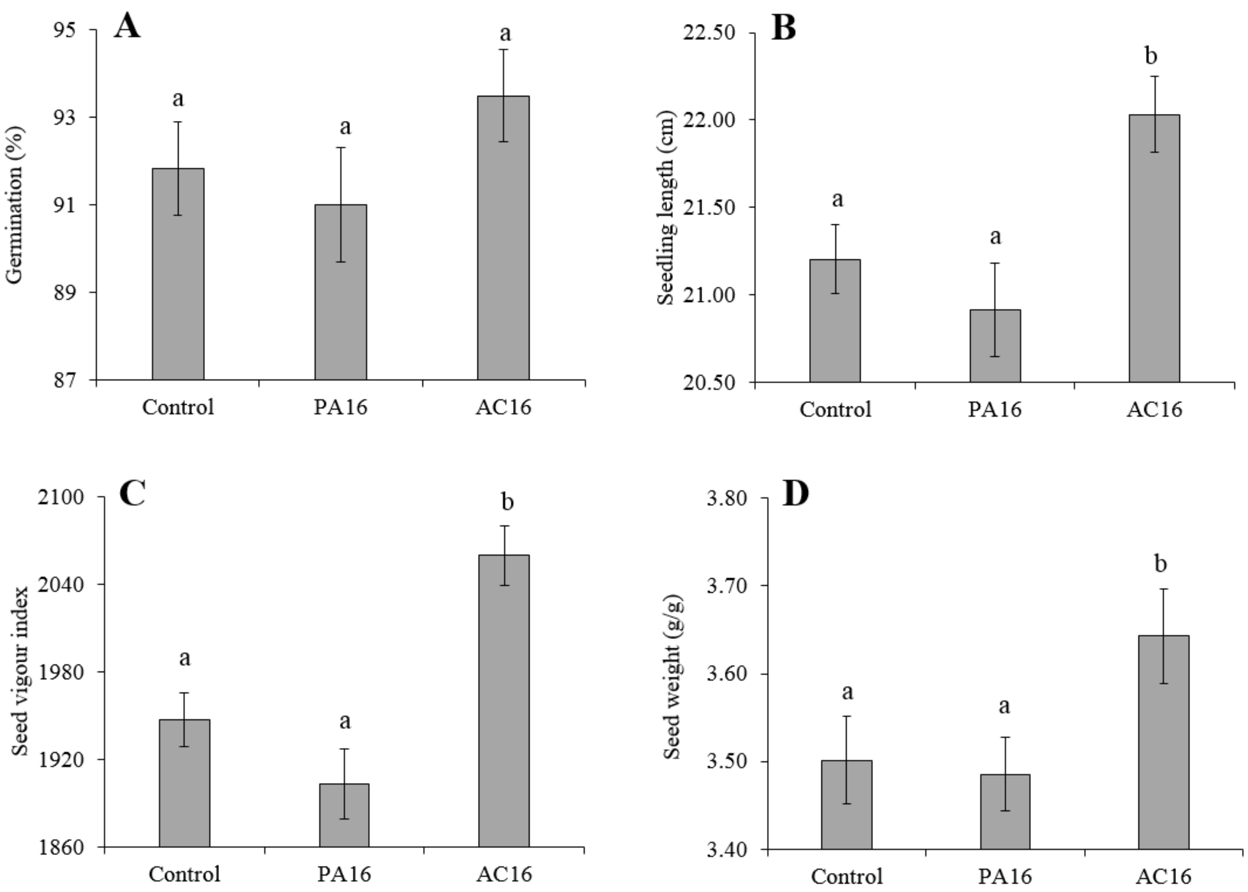

Fig. 4. The germination percentage (A), seedling length (B), seed vigour index (C) and seed weight (D) of uncoated (Control), 16 mL polyacrylamide (PA16) coated and 16 mL agar/ι-carrageenan (AC16) coated Durum wheat seeds under 40% moisture level. Different lowercase letters represent significant (p<0.05) differences according to the Tukey's test

Comparison of Growth Response between Polyacrylamide and Agar/ι-Carrageenan Coated Seeds at 40% Moisture Level

The mean values for *G*, *SL* and *SVI* results of AC16 coated seeds were higher than the mean values of PA16 coated seeds in all the data collected as presented in Fig. 4. It was also noticed that the level of abnormal seedlings (colour and appearance abnormalities as per ISTA guidelines) in the PA16 samples were higher than the control samples, while AC16 coated seeds had the lowest number of abnormal seedlings. However, statistical analysis revealed that the difference in the germination percentage between the control, PA16 and AC16 samples was not significant (p>0.05; Fig. 4A).

A significant difference was found, however, in the *SL* of AC16 coated seeds ($F_{2,267}$ = 6.42, P = 0.002; Fig. 4B). The AC16 sample had the longest shoot and root length compared to the control and PA16 coated seeds.

The beneficial effect of the AC16 coated seeds was mostly reflected in the *SVI* values where the seed vigour was much higher than the control and the PA16 coated seeds ($F_{2, 267}$ = 14.74, p<0.005; Fig. 4C).

Difference in Seed Weight

The *SW* was calculated by dividing the fresh weight of the seeds at the end of the germination test by the initial dry weight of the seeds before the test (refer to section 2.2.). Statistical results showed that the seed weight ratio of the AC16 seed was significantly higher than that of the PA16 samples ($F_{2,69}$ = 3.17, P = 0.048; Fig. 4D). No statistically significant difference was observed between the control and the PA16 samples.

Discussion

Effect of Coating Types and Volumes on Seed Germination at Ideal Growth Condition

The type of SAP seed coatings can be adapted depending on the purpose of the application; to delay or speed up germination, inhibit rot, control pests, fertilise, or bind the seed to the soil. In most cases SAP coatings on seeds provide more efficient imbibition of water prior to germination and it is one of the reasons for improved seedling growth. Ideally, the polymer coating absorbs water from the surrounding soils and holds it at the seed surface, thus increasing both the germination speed and the total number of germinated seeds (Akelah, 2013).

From this study, it was found that the PA coating (refer to Table 3) on Durum wheat seeds favoured faster germination at 80% moisture level. In terms of *RE*, the germination rate of the untreated seed was significantly lower than all the other levels of PA coated seeds. This suggests that the PA coating helped to activate early germination and to promote breaking dormancy under favourable growth conditions. PA gels are known to improve water-retention capacity of soil, enhance root development, regulate plant growth and improve soil cluster structures (Akelah, 2013). However, PA is thought to have no direct nutritional effect on plants, but thought to improve intake of nutrients from the soil to the plants more effectively (Seybold, 1994), suggesting that the PA coatings improved seed germination mainly through improvement in the water absorption by seeds (Fig. 3C).

The findings based on our results demonstrated that the AC coating (refer to Table 4) on seeds encouraged more root and shoot development than the increase in germination capacity. This suggested that the AC coating acted in a different way to the PA coating. The AC coating is composed of sugar based carbohydrates with trace elements such as Mg, K, Ca, Na and S as part of its chemical composition (Campo *et al*., 2009; Kelco, 2001; Prajapati *et al*., 2014). These may affect the seed growth by providing additional nutrition, or influence biological processes such as enzyme activity that promote root/shoot development (Njira and Nabwami, 2015).

In the case of *G*, the PA coating had a better effect on the seeds than the AC coating (Table 3 and 4). The *RE* and *SL* were significantly higher when using the AC coating under ideal growth condition (80% moisture). The effect was thought to be selective to the development of root/shoot cells. This was explained earlier as the possibility of the AC coating having higher levels of beneficial trace elements in the polymer compared to pure (< 0.05%, AAm) PA coating.

In terms of coating volume, a significant difference in *SVI* was found between the 2 and 16 mL coated seeds with PA coating (Table 3). In general the higher the coating volume, the greater the effect on the growth of the seeds for PA coated seeds.

The mean values of PA8 coated seeds tended to drop considerably compared to PA4 and PA16 coated samples in all parameters. Although no statistically significant difference was found, it was still worth noting that PA8 sample in particular seemed to inhibit the germination capacity and the growth of the seeds. Based on this observation the coating volumes that provided a positive effect on the seeds were: 4 and 16 mL for the PA coating. In case of the AC coating, as the coating volume increased the mean values for *G* and the *GSI* reduced. However, the mean values of *SL* and *SVI* showed an increase.

We hypothesise that the reduction in *G* for the AC coated seeds was caused by an increase in the coating thickness, since hydrogel coating can sometimes reduce the germination percentage and the speed due to reduced aeration around the seeds (Landis and Haase, 2012). The effect of increased coating thickness can be further supported by the observed growth promoting effect on the seeds. The amount of trace elements present in the coating would increase and become more available to the seeds if the coating thickness increased.

The *SVI* was used to decide the best coating volume for further investigation since the parameter is based on multiplication of *G* and the corresponding *SL*. The samples selected for further comparison of seed growth at low moisture level were the PA16 and AC16 samples.

Effect of Seed Coatings on Germination Performance under Low Moisture Level

Seed growth between PA16, AC16 and control (uncoated seeds) was compared at 40% moisture level (drought simulated condition) as shown in Fig. 4. No significant difference was found in the *G* between the samples (Fig. 4A). Whilst the observed differences were not of statistical significance, the higher mean germination percentage of AC16 coated seeds than those of the PA16 and the control samples appear to be of biological significance. In terms of *SL*, *SVI* and *SW* (Fig. 4B-4D), the AC16 sample performed significantly better than the PA16 and the control samples. In general the PA16 sample performed worse than the uncoated samples.

The results support that there was an advantage in using our natural blended hydrogel coating on the seeds over the synthetic PA coating under drought simulated conditions. The AC coating has helped promote faster seedling growth under both ideal and less than ideal conditions by promoting root/shoot development of seeds. This characteristic was also confirmed by the increase in *SW*.

It was thought that the increase in *SW* (Fig. 4D) was due to an increase in biological mass or an increase in water content of the seedlings. Since both PA and AC coatings are based on SAPs which have a strong capacity to absorb water, the two samples (PA16 and AC16) were both expected to perform similarly in drought conditions. However, the mean *SW* of the PA16 sample was slightly lower than the mean *SW* of the control, which indicated that the seed weight gain was less likely due to the increase in water absorption by the seeds. The weight gain of seeds was thought to be more likely caused by the increase in seedling biomass. Further studies are required to investigate the reason behind the weight gain of the fresh seeds.

Conclusion

The synthetic PA coating on Durum wheat seeds showed some healthy seed growth effect under ideal growth conditions (80% moisture level). The improvement in seedling performance was focused on the *RE*, *GSI* and *SVI* compared to the uncoated seeds. However, the PA coating did not show any significant improvement in less than ideal growth conditions.

In drought simulated conditions (40% moisture level), the AC coating showed a significant improvement in seed growth performance when compared to both the PA coated seeds and the uncoated seeds. In fact the PA coating had a negative effect on the seeds when compared to the uncoated seeds in drought simulated condition, showing low *G*, shorter *SL*, low *SVI* and lowest *SW*. The beneficial effect of the AC coating on the seeds was particularly observed in the improvement in *SL*, increased biomass and higher *SVI*.

From the study it was found that the natural AC hydrogel coating on Durum wheat seeds showed better potential to promote seed growth under drought stress than the synthetic PA coatings. It has also been shown that the AC coating performed better than the uncoated seeds.

In conclusion the novel AC hydrogel coating showed an advantage over the synthetic PA coating in terms of seedling growth under drought stress, material safety and production cost. Further up-scaled investigations are being carried out in a greenhouse for the growth promoting effect of AC hydrogel coatings and it is anticipated that this natural seed coating can contribute to future food sustainability.

Acknowledgement

This project was supported by the Athlone Institute of Technology President's Seed Fund 2014 and Agri Aware, an Irish charitable trust funded by the farming and agri-food industry. We would like to thank the Department of Agriculture, Food and the Marine (Backweston, Celbridge, Co. Kildare, Ireland) for their seed testing facilities and RAGT semenses (France) for their generous supply of Durum wheat seeds.

Author's Contributions

All authors contributed equally to this work in the design of experiment.

Noreen Morris and Clement Higginbotham: Developed the formulation of hydrogels.

Maya Hotta: Conducted the seed analysis.

James Kennedy: Performed statistical analysis on the collected data. All authors discussed the results and implications and commented on the manuscript at all stages.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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