PLANT GROWTH REGULATORS IN SEED COATING AGENT AFFECT SEED GERMINATION AND SEEDLING GROWTH OF SWEET CORN

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Abstract. To evaluate the potential effectiveness of plant growth regulators in improve seed germination and seedling vigor when applied during seed coating in sweet corn. 6-benzylaminopurine (6-BA), 1-naphthalene acetic acid (NAA), brassinolide and gibberellic acid (GA_3) were added as seed coating agent, and seed germination, antioxidant capacity and seedling vigor of sweet corn were investigated. The results showed compared with the use of coating agent alone, plant growth regulators improved seed vigor and germination, especially GA_3 (200 and 250 mg L^{-1}) and 6-BA (20 and 40 mg L^{-1}) were added. Furthermore, 200 mg L^{-1} GA_3 treatment improved seed germination and antioxidant capacity and resulted in sweet corn seedlings with a better appearance. The results observed indicated plant growth regulators might be valuable agents in sweet corn seed coating.
Keywords: *sweet corn, gibberellic acid (GA_3), 6-benzylaminopurine(6-BA), seed coating agent, seed vigor*

Introduction

Sweet corn hybrids carrying the *shrunken 2 (sh2*) gene, or supersweets, are extensively planted worldwide (Parera, 1990). Laughnan (1953) studied the effects of the *sh2* gene on carbohydrate reserves in maize endosperm and found that *sh2* endosperm stored less starch than normal types and possessed approximately 10-fold higher levels of total soluble sugars, with most of this increase being attributable to sucrose. Because of the higher levels of sugar in endosperm and a high sugar retention after harvest, sweet corn with the *sh2* gene provides superior quality and consumer appeal and permits longer transport and processing times (Duan, 1997).

Despite these superior features, the commercial acceptance and widespread use of *sh2* hybrids has been limited by poor seed quality. This poor seed vigor has been attributed to various factors, such as an insufficient nutrient supply during seed germination due to the low starch concentration. In addition, the higher imbibition rate of *sh2* kernels that leads to severe solute leakage increases susceptibility to physical damage and seed- and soil-borne diseases (Garwood et al., 1976; Styer et al., 1983). As a result, the yield and profitability of sweet corn possessing the *sh2* gene is hindered by poor seed vigor, as reflected in decreased emergence, poor seedling vigor, and erratic

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stand uniformity (Tracy, 1989; Parera et al., 1991).

Although various seed treatments, including fungicide treatment, pre-sowing hydration and bio-priming, are effective for improving sweet corn seed germination and seedling growth (Bennett et al., 1987; Tracy, 1989; Callan et al., 1990; Wilson et al., 1992; Hartz et al., 1995; Zhang et al., 2007), these methods have not been used on a commercial scale. As *sh2* hybrid corn is becoming a major commercial sweet corn genotype, seed coating technology that enhances seed value and promotes the mechanization of the planting process has attracted increasing attention. According to one study, the performance and physical properties of rice seeds are improved by coating them with liquid-based polymeric adhesives (Zeng et al., 2009). In addition, the oxygen provided to rice seeds planted under anoxic or near-anoxic soil conditions is increased when seeds are coated with peroxide compounds (Baker et al., 1987; Sono et al., 1991). Furthermore, germination and survival rates of seeds under adverse environmental conditions are promoted by coating with polymers incorporating pesticides (Taylor et al., 2001; Manjunatha et al., 2008). In sweet corn, however, seed germination is inhibited by coating with either polymers alone or polymers incorporating pesticides (Ikekawa et al., 1991; Lan et al., 2008). Plant growth regulators are active ingredients in coating agents, but their effects on seed coating have rarely been investigated. A In this study, we therefore conducted lab experiments to investigate the effects of plant growth regulators 6-benzylaminopurine (6-BA), 1-naphthalene acetic acid (NAA), brassinolide (BR) and gibberellic acid (GA_3) in seed coating agents on seed germination and seedling growth of sweet corn.

Materials and Methods

The study was conducted at the Crops Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong, China, during the summer of 2013.

Preparation of seed coating agent

Seed coating agent containing no active ingredients was provided by Incotec (Beijing, China). A GA_3 stock solution was prepared by dissolving 0.125 g of GA_3 in 1 mL absolute ethyl alcohol, followed by dilution with water to 250 mL and pH adjustment to 6.8–7.0. For use in subsequent experiments, this stock solution was diluted with water to give concentrations of 50, 100, 150, 200 and 250 mg L^{-1}. NAA (10, 20, 40, 60 and 80 mg L^{-1}), 6-BA (20, 40, 60, 80 and 100 mg L^{-1}) and BR (5, 10, 15, 20 and 25 mg L^{-1}) solutions were prepared in a similar fashion. The different plant growth regulator solutions were mixed with the Incotec seed coating agent in a 1:4 (v/w) ratio.

Seed coating treatments

The supersweet corn cultivar ‘Zhengtian 68’ bred at the Crops Research Institute, was used as seed material. All seeds were film-coated by hand. Seeds and seed coating agents with plant growth regulators were poured into a large plastic bag in a 1:50 (w/w) ratio. The bag was tightly closed and shaken to ensure even distribution of seed coating agents on seeds. The coated seeds were air-dried at room temperature for 2 h and then stored at 4 °C for 1 month.

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Germination testing

Samples comprising 150 seeds per treatment were placed on three Petri dishes (50 seeds per dish) and incubated in a growth chamber under controlled conditions (25–28 °C, 12-h photoperiod and 80–85 % relative humidity). Three replications were used for each treatment. Two controls were also set up: uncoated seeds (CK1) and seeds coated with coating agent without plant growth regulators (CK2). The number of germinated seeds was recorded daily for 1 week, and root and shoot lengths were measured 7 days after sowing. Germination potential, germination rate, germination index and vigor index were calculated according to Crop Seed Inspection Procedures of the National Standard of the People's Republic of China (GB/T 3543.1-1995) as follows: Germination rate (%) = (number of germinated seeds 7 days after sowing / total seed number) × 100 %; Germination potential (%) = (number of germinated seeds 3 days after sowing / total seed number) × 100 %; Germination index (GI) = ∑(Gt/Dt), and Vigor index = GI × S, where Gt is the number of germinated seeds on day *t*, Dt is the number of germination days, and S is seedling weight (in g).

Plant sampling and enzyme activity measurements

Treated and control seeds were sown in 10-cm diameter plastic containers and germinated in a growth chamber under controlled conditions (25–28 °C, 12-h photoperiod and 80–85 % relative humidity). Ten days after germination, fresh leaves from each treatment were sampled in liquid nitrogen, ground into a paste in an ice bath with 4 mL of 0.05 M phosphate buffer (pH = 7.8), transferred to a 10-mL centrifuge tube and centrifuged at 7,000 × *g* for 20 min. The resulting supernatant fluid was stored at −80 °C for measurement of superoxide dismutase (SOD), peroxidase (POD), malondialdehyde (MDA) and catalase (CAT) enzyme activities.

SOD activity was assayed by measuring the ability of the solution to inhibit the photochemical reduction of nitroblue tetrazolium following the method of Stewart et al. (1980). CAT activity was measured as the decline in absorbance at 240 nm due to the decrease of extinction of H_2O_2 using the method of (PATRA et al. 1978). POD activity was determined using the method of (AMAKO et al. 1994). In particular, the absorbance change of brown guaiacol at 460 nm was recorded to calculate POD activity, with one unit of POD enzyme activity defined as the amount of enzyme causing an increase of 1.0 in absorbance in 1 min due to guaiacol oxidation. The level of leaf senescence was determined by measuring the amount of MDA following the method of (Vos et al., 1991). Absorbance was recorded at 532 nm, with measurements corrected for non-specific turbidity by subtracting the absorbance at 600 nm. MDA concentration was determined on the basis of the extinction coefficient.

Statistical analysis

Data were analyzed using SPSS 19.0. Data were presented as mean±SEM. One-way ANOVA followed by Tukey test was used to compare mean values of the groups.

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Results

Seed germination

After storage at 4 °C for 1 month, seeds from all coating treatments, except for 200 and 250 mg L^{-1} GA_3 and 10 mg L^{-1} NAA, displayed significantly lower germination rates and germination indexes compared with CK1. The maximum germination potential was observed from the 200 mg L^{-1} GA_3 seed coating treatment, with no significant differences in germination potential found between the control and any NAA and 6-BA treatments, BR treatments except for 10 mg L^{-1} and the 200 and 250 mg L^{-1} GA_3 treatments. Coating treatments with 200 and 250 mg L^{-1} GA_3 gave rise to statistically significant increases in the vigor index compared with CK1, while 150 mg L^{-1} GA_3 and 20 and 40 mg L^{-1} 6-BA produced no significant differences in vigor index compared with CK1. The other treatments, namely 50 and 100 mg L^{-1} GA_3, 6-BA higher than 60 mg L^{-1}, and all levels of NAA and BR, resulted in a significantly lower vigor index compared with that of CK1.

Compared with CK1, all germination indexes—germination rate, germination potential, germination index and vigor index—were significantly decreased by the CK2 treatment. In particular, 150, 200 and 250 mg L^{-1} GA_3 and 10 mg L^{-1} NAA coating treatments caused statistically significant increases in germination rate relative to those from CK2 treatments, with the 100 mg L^{-1} 6-BA treatment producing seeds with the lowest germination rate among those from all coating treatments. The CK2 treatment was responsible for the lowest germination potential, and a significant increase was observed from all coating treatments except for 50 and 100 mg L^{-1} GA_3. Compared with CK2, the germination index was significantly increased by coating treatments of 200 and 250 mg L^{-1} GA_3, 20, 40 and 60 mg L^{-1} 6-BA and 10 mg L^{-1} NAA; no significant differences were observed as a result of the other coating treatments. Seed coating treatments of 200 and 250 mg L^{-1} GA_3 and 20 and 40 mg L^{-1} 6-BA produced seeds with statistically significantly higher vigor indexes compared with those from CK2 treatments. Higher levels of NAA (>10 mg L^{-1}) and 6-BA (>60 mg L^{-1}) and low levels of GA_3 (50 mg L^{-1}) significantly decreased the vigor index relative to CK2. No significant difference was observed after treatment at any BR level or with 10 mg L^{-1} NAA or 100 or 150 mg L^{-1} GA_3 (*Table 1*).

After storage for 2 months, germination indexes of sweet corn seeds were all decreased compared with values recorded after 1-month storage. The effects of seed coating treatments with different plant growth regulators all showed the same trends after storage for 2 months as those observed after 1 month (*Appendix 1*).

Table 1. *Effect of plant growth regulators in seed coating agent on seed germination of sweet corn*

Treatment (mg L^{-1})	Germination rate (%)	Germination potential (%)	Germination index	Vigor index
CK1	93.33±0.88a	59.67±0.33abc	30.39±0.24a	6.38±0.05B
CK2	78.00±1.73def	42.33±1.76i	24.76±0.70ghi	5.45±0.15CDE
GA_3-50	84.00±2.89bcde	47.33±0.88hi	25.29±0.41fgh	4.55±0.07GH
GA_3-100	85.00±2.08bcd	48.00±1.73ghi	25.66±1.00efgh	5.64±0.22CD
GA_3-150	85.67±2.03bc	51.67±1.67efgh	26.73±0.76cdefg	5.88±0.17BC
GA_3-200	89.67±0.33ab	61.67±1.20a	29.12±0.50ab	7.28±0.12A
GA_3-250	87.67±0.67ab	57.00±0.58abcde	28.71±0.25abcd	7.46±0.06A
6BA-20	84.00±2.52bcde	60.67±1.20ab	27.72±0.57bcde	6.37±0.13B
6BA-40	82.67±0.88bcde	60.33±0.88abc	27.76±0.41bcde	6.38±0.09B

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6BA-60	82.33±2.03bcde	58.33±1.45abcd	27.36±0.50bcdef	4.93±0.09EFG
6BA-80	72.00±1.00fg	56.00±2.08abcde	24.82±0.34ghi	4.22±0.06H
6BA-100	67.33±3.53g	49.33±1.67fgh	22.60±0.77i	3.62±0.12I
NAA-10	86.67±2.33ab	60.33±1.20abc	28.87±0.48abc	5.49±0.09CDE
NAA-20	79.00±2.00cdef	60.00±0.58abc	26.61±0.61defg	4.79±0.11FG
NAA-40	77.33±4.18ef	58.33±2.19abcd	26.46±1.35defg	4.23±0.22H
NAA-60	74.00±1.53fg	55.67±1.86abcde	25.32±0.67fgh	4.05±0.11HI
NAA-80	74.00±2.52fg	54.67±1.33bcdef	25.18±0.82fgh	3.52±0.11I
BR-5	77.33±3.48ef	57.33±2.91abcde	25.17±0.83fgh	5.54±0.18CD
BR-10	77.67±0.33def	52.33±4.33defgh	24.47±0.78ghi	5.14±0.16DEF
BR-15	71.67±3.28fg	56.00±2.08abcde	23.96±1.04hi	5.27±0.23DEF
BR-20	76.67±1.67ef	54.00±3.61cdefg	24.57±0.60ghi	5.65±0.14CD
BR-25	77.67±0.33def	55.00±1.15bcdef	24.88±0.39gh	5.22±0.08DEF

Note: different lowercase and uppercase letters are used to indicate values that are significantly different at $p < 0.05$ and $p < 0.01$, respectively

Root and shoot lengths

Compared with CK1, all treatments caused significantly lower root lengths. The 100–250 mg L^{-1} GA_3 treatments significantly increased shoot lengths, while no significant difference in shoot length was observed from 50 mg L^{-1} GA_3 or 20 or 40 mg L^{-1} 6-BA. Higher levels of 6-BA and BR, as well as all levels of NAA, had a significant inhibitory effect on shoot length (*Table 2*).

Table 2. *Effect of plant growth regulators in seed coating agent on root and shoot lengths of sweet corn seedlings*

Treatment	Root length（cm）	Shoot length（cm）
CK1	18.44±0.08A	8.88±0.05DE
CK2	16.86±0.12EFG	8.10±0.08FG
GA_3-50	16.74±0.16FG	8.75±0.03E
GA_3-100	17.15±0.03DEF	9.86±0.01C
GA_3-150	17.68±0.17BC	10.55±0.07B
GA_3-200	17.98±0.12B	11.56±0.04A
GA_3-250	17.40±0.05CD	10.76±0.06B
6BA-20	16.10±0.14I	9.03±0.08D
6BA-40	15.24±0.10J	8.70±0.03E
6BA-60	13.25±0.05L	7.68±0.03IJ
6BA-80	10.99±0.08M	7.02±0.07L
6BA-100	10.26±0.11N	6.70±0.04M
NAA-10	17.29±0.04CDE	7.90±0.09GH
NAA-20	16.94±0.03EFG	7.56±0.06JK
NAA-40	16.25±0.04HI	7.44±0.05K
NAA-60	14.62±0.17K	7.36±0.06K
NAA-80	13.54±0.04L	7.11±0.06L
BR-5	16.63±0.03GH	8.93±0.08DE
BR-10	16.15±0.17I	8.31±0.07F
BR-15	15.54±0.13J	8.24±0.01F
BR-20	14.61±0.03K	8.13±0.04F
BR-25	13.46±0.18L	7.83±0.04HI

Note: different uppercase letters are used to indicate values that are significantly different at $p < 0.01$

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Compared with CK2, treatments with 150, 200 or 250 mg L^{-1} GA_3 dramatically increased root lengths. No significant differences in root lengths were observed following treatment with coating agents containing 10 or 20 mg L^{-1} NAA, whereas the other treatments, namely, lower levels of GA_3 (50 and 100 mg L^{-1}), higher levels of NAA (40, 60 and 80 mg L^{-1}) and all levels of BR and 6-BA, significantly inhibited root lengths (*Table 2*). A significant enhancement in shoot length was produced by all GA3 treatments as well as 20 and 40 mg L^{-1} 6-BA and 5 mg L^{-1} BR treatments. No significant differences in shoot lengths were observed following 10, 15 or 20 mg L^{-1} BR treatments, while a significant decrease was observed after treatments involving 60, 80 or 100 mg L^{-1} 6-BA, 25 mg L^{-1} BR and all NAA levels (*Table 2*).

According to above data, coating treatments with 200 mg L^{-1} GA3 gave rise to statistically significant increases in the vigor index and 100 mg L^{-1} GA3resulted in a significantly lower vigor index compared with that of CK1. Sweet corn seedlings at 7 days after sowing from seeds subjected to CK1, CK2, 200 mg L^{-1} GA3 and 100 mg L^{-1} 6-BA coating treatments are presented in *Fig. 1*. As is obvious from the figure, seedlings from the 200 mg L^{-1} GA3 treatment had an outstanding appearance that contrasted with those from 100 mg L^{-1} 6-BA.

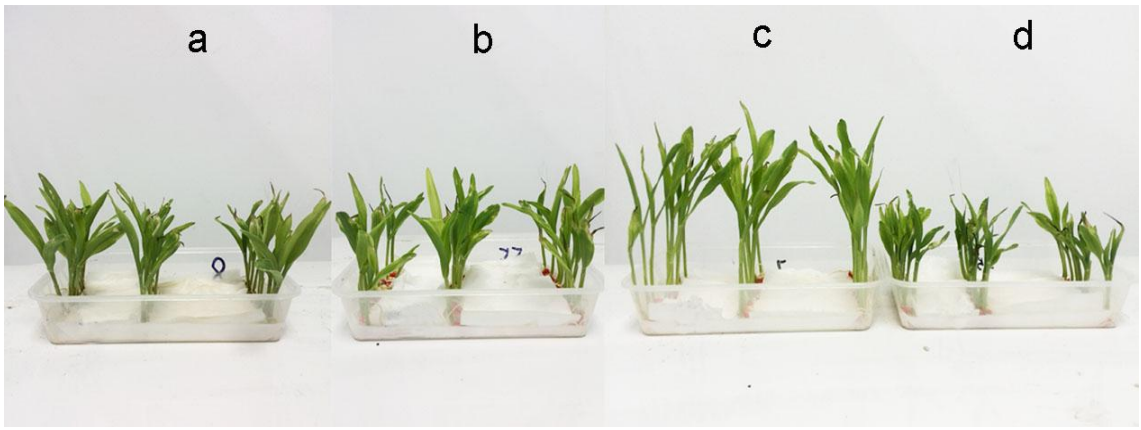

Figure 1. *Appearance of sweet corn seedlings at 7 days after sowing from seeds subjected to different seed coating treatments. a: uncoated seeds (CK1); b: no plant growth regulator in the coating agent (CK2); c: 200 mg L^{-1} gibberellic acid (GA_3); d: 100 mg L^{-1} 6-benzyladenine (6-BA)*

Physiological resistance

A pot experiment was conducted to investigate the physiological resistance of seedlings from selected treatments (CK1, CK2, 200 mg L^{-1} GA_3 and 100 mg L^{-1} 6-BA). Compared with CK1 and CK2, 200 mg L^{-1} GA_3 was respectively found to significantly increase SOD activities by 5.69 and 6.83 %, POD activities by 26.05 and 4.83 % and CAT activities by 18.98 and 14.67 % and to significantly decrease MDA content by 29.26 and 39.69 %. With respect to SOD, POD, and CAT activities and MDA content, the following trends were observed: SOD activity, 200 mg L^{-1} GA_3 > 100 mg L^{-1} 6-BA > CK1 > CK2; POD activity, 100 mg L^{-1} 6-BA > 200 mg L^{-1} GA_3 > CK2 > CK1; CAT activity, 200 mg L^{-1} GA_3 > 100 mg L^{-1} 6-BA > CK2 > CK1; and MDA content, CK2 > CK1 > 100 mg L^{-1} 6-BA > 200 mg L^{-1} GA_3.

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Discussion

The seed coating process involves application of pesticides, fertilizers, oxygen agents or growth regulators to seeds to resist diseases and pests, and to promote seed germination and seedling growth (Taylor et al., 2001; Zhang et al., 2007). Previous studies have shown seed coating technology to be an effective approach for improving seed germination and seedling growth of crop plants (Taylor et al., 2001). In rice, seed coating improves the performance and physical properties of seeds, especially under adverse environmental conditions (Baker et al., 1987; Sono et al., 1991; Tylor et al., 1998; Manjunatha et al., 2008; Zeng et al., 2009). Seed coating with salicylic acid, paclobutrazol or humic acid has a positive effect on seed germination and seedling growth in maize (Lan et al., 2008; Wang et al., 2010; Zhu et al., 2013). Boschi et al. (2014) reported the effect of 6-BA on the germination and growth of seeds of *Ginkgo biloba* and suggested that seed immersion with 2.5 ppm of 6-BA performed.

Sweet corn, with its naturally poor seed vigor, differs from other maize types (Harris et al., 1989). A key agricultural objective to increase sweet corn yields is achievement of rapid, uniform germination and seedling emergence (Rajjou et al., 2012). However, we found that a coating treatment lacking plant growth regulators inhibited seed germination and vigor (*Table 1*), which was consistent with previous studies (Ikekawa et al., 1991; Lan et al., 2008). This inhibition may be due to physical damage caused by seed coating.

Experimental coating with different concentrations of 6-BA and NAA showed that low concentrations of these plant growth regulators promoted seed germination, whereas high concentrations inhibited it (*Table 1*). Suitable concentrations of 6-BA (20–40 mg L^{-1}) and NAA (10–20 mg L^{-1}) were beneficial for rapid, uniform germination and seedling emergence. BR is an important phytohormone that plays an important role in various aspects of plant growth and development, including seed germination (Wilen et al. 1995; Dhaubhadel et al. 1999; Khripach et al. 2000; Miransari et al. 2014). Although seed immersion with BR has been found to promote seed germination in maize (Zou, 2002), we observed no obviously positive effect in coating treatments incorporating BR in the coating agent.

We found that coating using coating agent alone inhibited seed germination, as reflected by decreased germination rate, germination potential, germination index and vigor index. In contrast, coating treatments incorporating 200 and 250 mg L^{-1} GA_3 maintained a high germination rate, germination index and germination potential similar to CK1 and significantly increased vigor index and shoot length compared with CK1 (Tables 1 and 2). These positive effects may be due to the roles of GA in breaking dormancy and promoting seed germination and stem elongation (Gurdiola, 1996). In addition, 200 mg L^{-1} GA_3 significantly increased SOD, POD and CAT activities and decreased the accumulation of MDA content compared with CKs (*Figs. 1* and *2*). This result implies that GA_3 coating treatments improve seed germination and seedling physiological resistance by increasing SOD, POD and CAT activities in seedlings and by reducing membrane damage during the coating process.

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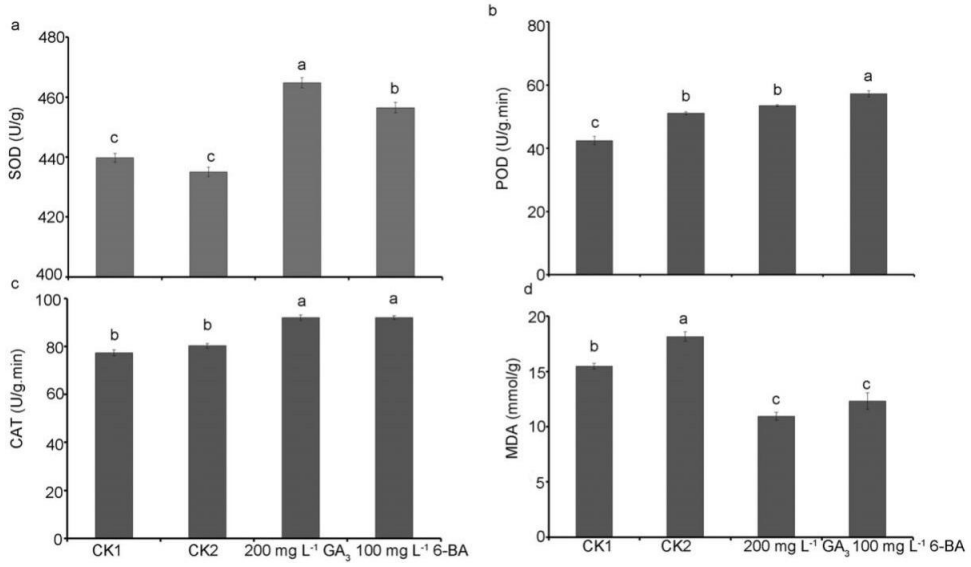

Figure 2. *Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and malondialdehyde (MDA) contents of sweet corn seedlings at 10 days after sowing. Different lowercase letters indicate values that are significantly different at $p < 0.05$*

Conclusion

In this study, we found seed coating using coating agent alone inhibited seed germination, whereas, coating with suitable concentrations of 6-BA (20–40 mg L^{-1}), GA_3 (200 mg L^{-1}) and NAA (10–20 mg L^{-1}) were beneficial for rapid, uniform germination and seedling emergence in sweet corn. Which implied it is effective approach to improve seed germination through developing seed coating incorporated with suitable concentrations plant regulators (6-BA, GA_3, NAA) in sweet corn.

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APPENDIX

Appendix 1. *Effect of plant growth regulators in seed coating agent on seed germination of sweet corn after storage of seeds for 2 months*

Treatment (mg L^{-1})	Germination rate (%)	Germination potential (%)	Germination index	Vigor index
CK1	91.00±1.00A	50.67±0.67A	27.45±0.19A	5.49±0.73A
CK2	56.67±0.88DEF	28.33±0.67EFGH	16.71±0.34FGH	3.18±0.09E
GA_3-50	57.00±1.56EDF	34.67±0.33CD	17.57±0.51DEF	3.34±0.09DE
GA_3-100	58.00±1.15DE	36.00±1.00C	18.51±0.33CD	3.52±0.11E
GA_3-150	62.33±0.33BC	37.67±0.88BC	19.23±0.19C	3.85±0.11C
GA_3-200	65.00±1.15B	40.67±0.67B	20.87±0.19B	4.17±0.06B
GA_3-250	62.23±0.33BC	37.00±1.00BC	19.32±0.26C	4.06±0.25BC
6BA-20	55.00±1.15EFG	29.67±1.33EFG	16.54±0.44FGH	3.31±0.14DE
6BA-40	53.67±0.67FGH	27.00±0.58FGH	16.08±0.13GHI	2.89±0.11FG
6BA-60	50.33±0.88HIJ	25.67±1.20GH	15.29±0.49HIJK	2.60±0.10H
6BA-80	48.67±0.67IJK	24.67±0.88H	14.64±0.11IJKL	2.34±0.18I
6BA-100	42.67±1.45M	21.00±1.00I	12.73±0.52M	1.78±0.48J
NAA-10	59.00±1.00CD	38.33±1.20BC	18.22±0.46CDE	3.28±0.13DE
NAA-20	57.33±0.67DEF	32.00±1.15DE	17.09±0.30EFG	2.91±0.09F
NAA-40	53.67±0.67FGH	29.00±1.15EFG	15.66±0.28GHIJ	2.66±0.08GH
NAA-60	51.33±0.88GHIJ	28.33±0.67EFGH	15.27±0.24HIJK	2.44±0.05HI
NAA-80	47.67±0.33JKL	27.33±0.33FGH	14.37±0.15JKL	2.30±0.20I
BR-5	52.33±1.20GHI	31.33±0.67DE	16.19±0.38FGH	2.91±0.10F
BR-10	51.33±0.88GHIJ	30.00±0.58EFG	15.70±0.34GHIJ	2.67±0.09GH
BR-15	48.67±0.67IJK	29.67±1.33EFG	15.29±0.36HIJK	2.45±0.15HI
BR-20	46.33±0.67KLM	27.00±1.00FGH	14.18±0.33KL	1.98±0.05J
BR-25	44.33±1.20LM	25.00±1.00H	13.67±0.43LM	1.91±0.06J

Note: different lowercase and uppercase letters indicate values that are significantly different at $p < 0.05$ and $p < 0.01$, respectively. Values are the means of three biological replicates ± standard error. Different capital letters in each row indicate significant differences as determined by the analysis of variance, $p < 0.01$

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