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Effects of Super Absorbent Polymers and Gibberellin on Germination and Growth of alfalfa

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ABSTRACT

For proven effects of eco-friendly super absorbent polymer and gibberellin on germination and growth of alfalfa, taking the germination and growth test in the artificial climate box by adding the different amount of super absorbent polymer and gibberellin. Observed the germination potential, germination rate, shoot length, root length, fresh weight and dry weight, under the different experiment conditions. The test showed that the super absorbent polymer had a promoting effect on alfalfa. The property of slowly release water, water supply on super absorbent polymer could keep plants prolonging the survival rate in the case of insufficient water supply. It could improve the germination rate of the seed of alfalfa. Adding super absorbent polymer and gibberellin not only improved the survival rate, but also promoted the growth of root and shoot on alfalfa.

Key words: Alfalfa, Gibberellin, Seed germination, Seedling growth, Super absorbent polymer.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is known as an excellent forage that has the largest planting area and the highest economic benefits in China, mainly cultivated in northwest China, where is arid and semi-arid and desertification is serious in most areas. Water shortage and nutrient deficiency are two key factors that restricted crop production in these areas. Alfalfa valuable as fodder and for soil-building, it has a tenacious vitality and it is a good variety to improve soil. It can be planted in annual rainfall of 250 to 800 mm. The annual rainfall in much of northern China is about 500 mm and rainfall mainly distributed in July and August. And other seasons are almost arid, as a result that water for alfalfa growth is in shortfall.

Super absorbent polymer (SAP) is a material which can absorb hundred times more than itself. The research shows that super absorbent polymer has a function of absorbing water repeatedly (Wang *et al*., 2017). As a hydrogel, it can slowly release the water supply plant uptake after absorbing water expansion (Rabat *et al*., 2016). Enhancing the soil water retention can increase efficiency of water using of the crop (Guilherme *et al*., 2015). Hence, it is possible to solve the problem of water shortage and help alfalfa grow by the super absorbent polymer.

In addition, gibberellin is one of the most essential plant growth hormones. It can break the plant dormancy, promote the gene expression and elongation of the cells, increase the height of the plant by promoting the elongation of the stem, stimulate the growth of plant leaves and buds and the development of the flower, seed and fruit. It plays a vital role in the development of plants and has been proved to be in many plants (Tenreira *et al*., 2017).

Therefore, the purpose of our experiment is to conform it can solve the problem of water shortage and improve the survival rate of alfalfa and improve its growth condition by using super absorbent polymer and gibberellins.

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MATERIALS AND METHODS

Experimental materials

Medicago sativa L. Gannong No. 6 (Breeder supply), super absorbent polymer (lab-synthesized: we synthesized green environment-friendly salt-resistant high performance and low-cost SAP by grafting co-polymerization of incomplete neutralized acrylic acid (AA) onto a banana peel backbone in the presence of modified palygorskite clay.), double distilled water (lab-synthesized), gibberellins were provided by Zhongtai chemical reagent Co., Ltd, Shanghai, China.

Experimental designs

The experiment has 7 treatments, all of which are single-factor complete random design, distilled water (A), swelling water absorbent resin (B), 0.01% gibberellin solution (C), distilled water, covering method with 0.01% gibberellin solution regularly spraying alfalfa stem and leaf (D), distilled water, covering method with 0.01% gibberellin solution

regularly spraying root (E), swelling water absorbable resin, covering method with 0.01% gibberellin solution regularly spraying alfalfa stem and leaf (F), swelling water absorption resin, covering method with 0.01% gibberellin solution regularly spraying alfalfa root (G), each treatment three repeated experiments. A paper bed method was used for the germination experiment. A culture dish containing two filter papers was used as the germinating bed and 30 alfalfa seeds had been screened and the selection principle was that normal of alfalfa seeds was full and the outside green coating was not shedding. Constant weight was maintained by weighing method 24 h.

Before the experiment, the super absorbent resin was soaked sufficiently to make it absorb water and swell completely and then filtered for reserve. At the same time, the concentration of gibberellin solution is 0.01%. Artificial climate chamber (RQH-280C) sets the conditions for seed germination and seedling growth and the specific parameters are listed in Table 1.

Measurement

Germination rate and Germination potential are determined according to China Natiaonal Standard (GB/T5520-2011). Water consumption was measured by weighing method. The root length and shoot length were measured by meter rule. Then keep them becoming dry with filter paper, fresh weight. After measuring the fresh weight of root and shoot, made them dry in oven and recorded the dry weight. Determination of root activity of Alfalfa by TTC reduction.

Statistical analyses

Statistical analyses were processed using the computer software SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to compare the priming treatment effect and significant differences of means were separated using Duncan's test ($p< 0.05$).

RESULTS AND DISCUSSION

Germination rate

The effect of different treatments on the germination rate of alfalfa seeds can be clearly seen in Fig 1. Among them, the germination rate of each experimental group was significantly different from that of the control group A ($p< 0.05$). The germination rate of the C group was 93.3% in the whole group and the results of the experiment were obviously better than those of the control group A, B and D and the difference was significant ($p< 0.05$). At the same time, it was better than the other three groups of E, F and G.

Table 1: Setting parameters of the artificial climate chamber in the experiment.

Setting parameters	Part one	Part two
Temperature	19°C	24°C
Humidity	55%	40%
Light intensity	0	3
Time	720 min	720 min

The germination rates of E, F and G group were 88.9%, 91.1% and 87.8% respectively. The difference between the experimental results were not obvious ($p> 0.05$), but the germination rate of B and D groups was 76.7% and the difference was not significant ($p> 0.05$).

It can be seen from different experimental conditions that super absorbent polymers and gibberellin can promoted the germination of alfalfa seeds and the germination rate of the experimental group with super absorbent polymers or gibberellin all increased significantly. The different application modes of gibberellin had a promoting effect on germination rate, but the results were slightly different. Group C experiment was equivalent to directly soaking seeds with gibberellin and the result was better than spraying method. The main reason is that gibberellin has the advantage of slowing down the dormancy period of plant seeds and improving germination. Compared with the control group, the germination rate of the high water absorbent resin was more obvious than that of the control group and it was different from the effect of the plant hormone gibberellin. It indicated that the high water absorbable resin had a significant effect on the germination rate of alfalfa. At the same time, it was suggested that the germination rate of plants should be increased and the germination of the plants should be promoted. In addition to adding exogenous gibberellin, the substrate can also be replaced by adding super absorbent polymers to achieve similar results.

Germination potential

Germination potential is an important index for testing the quality of seeds. To a certain extent, the germination speed and the degree of uniformity of the tested seeds were shown. The higher the germination potential, the higher the seed

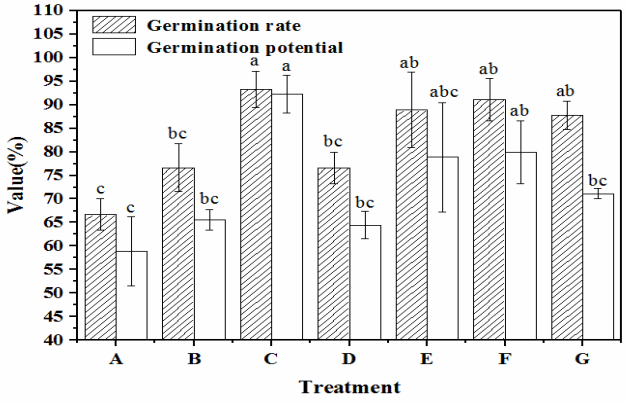

Fig 1: Germination rate and germination potential under different experimental treatments. (A) water. (B) swollen BPL-g-PAA/mPGS. (C) gibberellin solution. (D) water, sprinkle shoot regular with gibberellin solution. (E) water, sprinkle root regular with gibberellin solution. (F) swollen BPL-g-PAA/mPGS, sprinkle shoot regular with gibberellin solution. (G) swollen BPL-g-PAA/mPGS, sprinkle root regular with gibberellin solution. Each value represents the mean±SE (n=30). The different letters are significantly different at the 5% ($p\leq0.05$).

quality. The experimental results of the germination potential of alfalfa treated with different experimental groups are shown in Fig 1. Compared with the control experiment, the sprouting vigor was significantly improved by adding SAP or gibberellin. Among them, the germination potential of the control group A was 58.9% and that of C group and F group was 92.2% and 80% respectively ($p< 0.05$). There was no significant difference between group B (65.6%), group D (64.4%), group E (78.9%) and group G (71.1%) ($p> 0.05$). It is known from the experimental data that, due to the characteristics of gibberellin plant hormone, it can directly regulate the synthesis of enzyme and protein in plant cells. Therefore, the introduction of gibberellin can break the dormancy period, making the germination potential of alfalfa significantly improved. The application of SAP also obtained similar experimental result and also significantly improved the germination potential. The main reason is that the SAP can provide enough moisture for seed germination. The SAP absorbs water molecules and retains their three-dimensional network structure. This part of water will not evaporate directly and stores the water needed for seed germination. At the same time, studies have shown that the power of seed to absorb water is stronger than that of SAP. Therefore, the seeds can make good use of the water absorbed and preserved by SAP. The experimental results show that the addition of SAP not only has no toxic effect on the germination of alfalfa, but also has a similar promotion effect with plant hormone. In order to study the role of super absorbent polymer in the germination stage of plants, the quality of seeds was explained in this respect.

Water consumption

Fig 2 shows the water consumption under different treatments. From the graph, the minimum water consumption of group B was 211.6 g, which was lower than that of the control group. The rest of the experimental group was C group (233 g), D group (230.7 g), E group (234.9 g), F group (228.8 g) and G group (218.8 g) respectively. The water consumption of C group, D group and E group without adding SAP was significantly higher than those of B group containing SAP ($p< 0.05$). This is mainly because water consumption comes from two main aspects: plant consumption and water evaporation. According to other researchers, water absorbent resin can retain water well at high temperature and high pressure, which directly reduces the evaporation of part of the water and reduces water consumption. This can also fully explain the addition of super absorbent polymers, which can reduce water consumption very well, save water to a certain extent and optimize the preparation of water resources. The experiment group introduced gibberellin increased water consumption, probably because the synthesis of amino acids and enzymes in the plant cells was regulated by gibberellin and the whole process needed to consume water, which significantly increased the part of plant consumption. It can also be found that the water consumption of the experimental group containing the high water absorption resin and gibberellin is lower than that of the experimental group only with gibberellin, which also indicates that the excellent water absorption and water retention property of the SAP can effectively reduce the water consumption.

Root length and shoot length

The germ of the seed develops into a stem and leaf, the radicle develops into the root and the middle connecting root and stem is derived from the hypocotyl. The growth of plants depends largely on the growth of their roots and stems. Therefore, the effects of super absorbent polymers and gibberellin on the growth of roots and stems of alfalfa were studied under different treatments (Fig 3).

The data showed that compared with A group (3.9 cm), group B (4.7 cm), C group (3.3 cm), D group (8.2 cm) and E group (6.8 cm) showed significant differences ($P < 0.05$). The F group (4) and the G group (4.3), although the root length increased, were not obvious in the previous group and the difference was not significant (0.05). The application of SAP significantly increased root length and showed a strong promotion effect of super absorbent polymers. As for gibberellin, there were significant differences in the effects of different methods of introduction. The differences in the C group, D group and E group were significant ($p< 0.05$), while the experimental group with SAP and gibberellin relieved this difference and the difference was not significant ($p> 0.05$).

For the stem, compared to the control group A (1.5 cm), the other groups of B groups (1.8 cm), C group (2.7 cm), D group (2.7 cm), E group (2.9 cm) and F group (4 cm) showed significant differences. It indicated that gibberellin and super absorbent resin both showed obvious effects on promoting

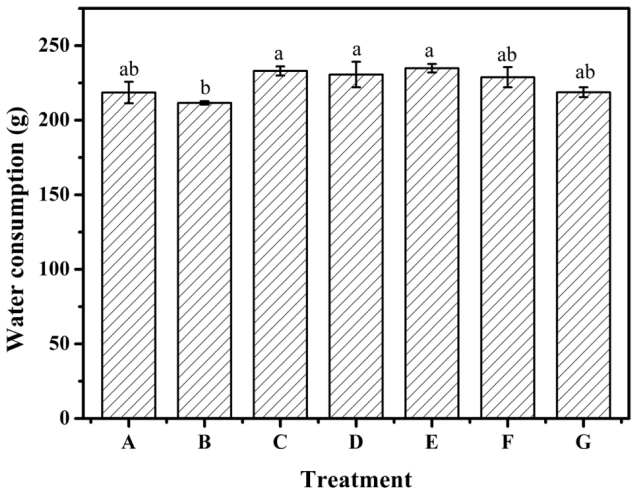

Fig 2: Water consumption under different tests. (A) water. (B) swollen BPL-g-PAA/mPGS. (C) gibberellin solution. (D) water, sprinkle shoot regular with gibberellin solution. (E) water, sprinkle root regular with gibberellin solution. (F) swollen BPL-g-PAA/mPGS, sprinkle shoot regular with gibberellin solution. (G) swollen BPL-g-PAA/mPGS, sprinkle root regular with gibberellin solution. Each value represents the mean±SE (n=30). The different letters are significantly different at the 5% ($p\leq0.05$).

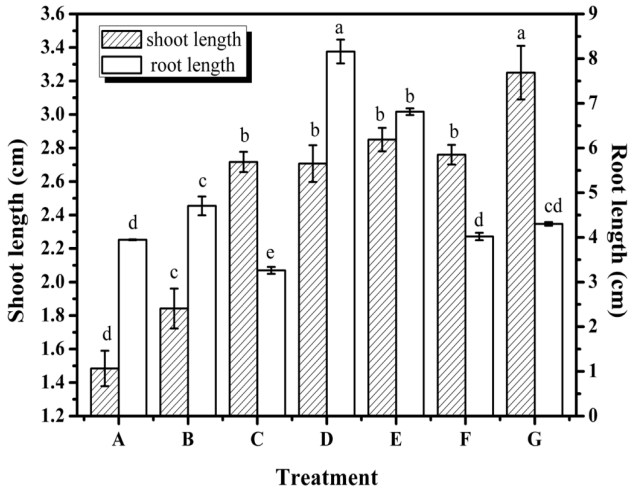

Fig 3: Shoot and root length under different experimental treatment. (A) water. (B) swollen BPL-g-PAA/mPGS. (C) gibberellin solution. (D) water, sprinkle shoot regular with gibberellin solution. (E) water, sprinkle root regular with gibberellin solution. (F) swollen BPL-g-PAA/mPGS, sprinkle shoot regular with gibberellin solution. (G) swollen BPL-g-PAA/mPGS, sprinkle root regular with gibberellin solution. Each value represents the mean±SE (n=30). The different letters are significantly different at the 5% (p≤0.05).

stem growth. Among them, the G group with high water absorbing resin and gibberellin was 3.3 cm, with the highest value.

The main reason for this difference is that the physiological function of the hormone depends not only on the concentration of the hormone in the plant tissue, but also to the sensitivity of the plant tissue itself to the response of the hormone stimulation. This difference is due to the mechanism of hormone influence. Hormones want to cause certain reactions and ultimately induce gene expression. First, they are combined with the receptor proteins that exist in the cytoplasm and on the cell membrane and then activate them. This may be due to the difference in the number of receptors in the roots and stems of alfalfa, the affinity of the receptor to gibberellin and the response ability of Alfalfa itself, which makes the sensitivity of the alfalfa different, which leads to the difference of the experimental results.

Biomass

As shown in Fig 4, the effects of different experimental conditions on biomass accumulation were studied respectively from fresh weight and dry weight. It can be seen from the map that the promotion SAP and gibberellin on fresh weight and dry weight is obvious and the difference is significant ($p < 0.05$). Compared with the control group, the accumulation of biomass is increased greatly.

No matter the fresh weight or dry weight of the root, the promotion effect of SAP is most obvious and the difference is significant ($p < 0.05$). This may be due to the contact between the root and the SAP and the expansion of the network segment of the three-dimensional structure of the swelling gel, the full exposure of the hydrophilic group and the interaction between the ions. It is used to stimulate the root of alfalfa directly and the stimulus response of the root is more obvious and the growth of the root is more vigorous. For stem, fresh weight or dry weight, the experiment showed that the best effect was promoted by gibberellin and super absorbent resin. Moreover, the experimental results caused by different introduction methods of gibberellins were not significantly different. The difference in data between these experimental groups was not significant ($p > 0.05$).

It can be seen that the accumulation of biomass was more obvious when gibberellin was applied. Because of its characteristics, such as increasing cell division, promoting

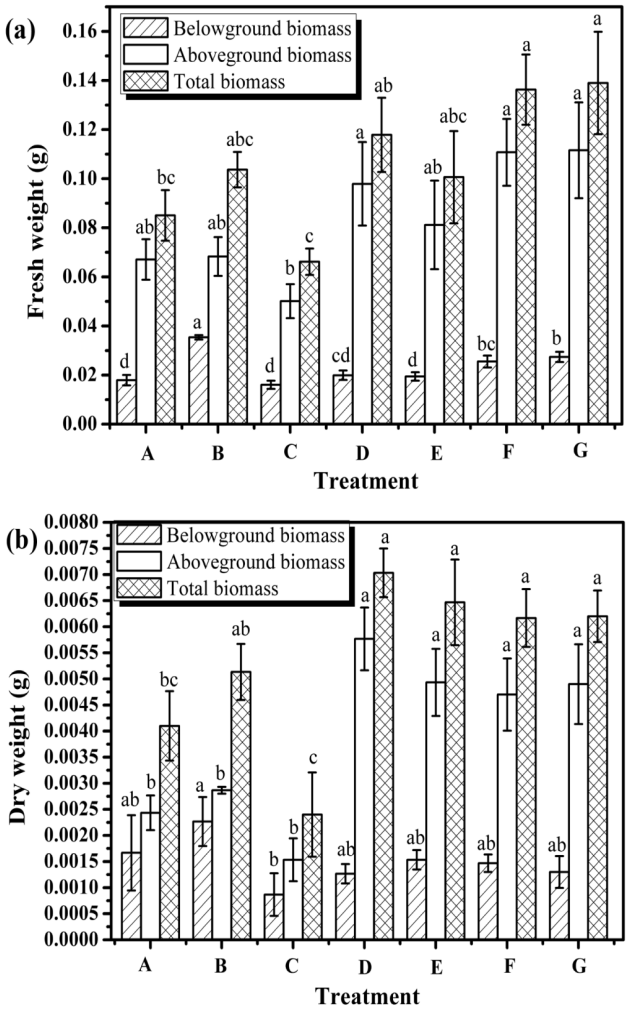

Fig 4: Belowground biomass, aboveground biomass and total biomass under different experimental treatment. (A) water. (B) swollen BPL-g-PAA/mPGS. (C) gibberellin solution. (D) water, sprinkle shoot regular with gibberellin solution. (E) water, sprinkle root regular with gibberellin solution. (F) swollen BPL-g-PAA/mPGS, sprinkle shoot regular with gibberellin solution. (G) swollen BPL-g-PAA/mPGS, sprinkle root regular with gibberellin solution. Each value represents the mean±SE (n=30). The different letters are significantly different at the 5% (p≤0.05).

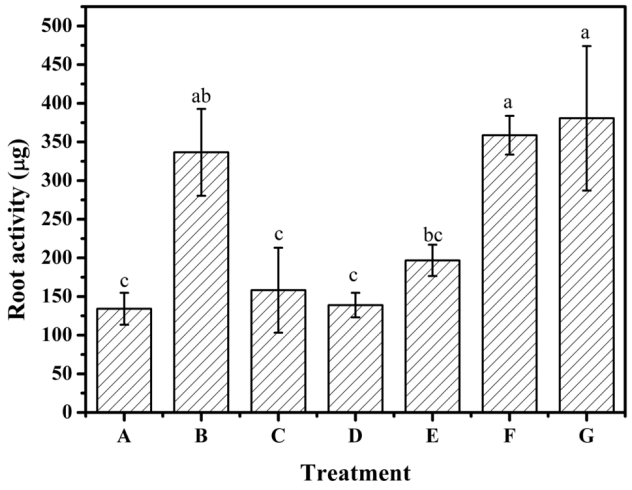

Fig 5: Root activity under different tests. (A) water. (B) swollen BPL-g-PAA/mPGS. (C) gibberellin solution. (D) water, sprinkle shoot regular with gibberellin solution. (E) water, sprinkle root regular with gibberellin solution. (F) swollen BPL-g-PAA/mPGS, sprinkle shoot regular with gibberellin solution. (G) swollen BPL-g-PAA/mPGS, sprinkle root regular with gibberellin solution. Each value represents the mean±SE (n=30). The different letters are significantly different at the 5% ($p \leq 0.05$).

cell infiltration and water absorption, it may lead to the growth of plant stems, which makes the accumulation of stem biomass increased obviously. And the biomass accumulation of SAP to root is more significant. The leading factor in this situation is the response to external stimuli. The different parts of alfalfa have different sensitivities, that is, the affinity of gibberellic receptor in different parts is different, which shows that the biomass accumulation of roots and stems is not uniform, or even very different.

Root activity

The growth status of the aerial part is directly affected by the root activity (Fang *et al*., 2018). Therefore, it is necessary to study the root activity. According to Fig 5, the effect of different experimental conditions on the root activity coefficient of alfalfa was studied. Among the groups of A, C, D and E, the differences in B, F and G were not obvious ($p > 0.05$) and the root vitality of the experimental group with the participation of SAP was significantly stronger ($p < 0.05$). It showed that it had an obvious effect on the root activity of alfalfa, but the effect of gibberellin was not significant, with the different methods of application, there was no significant difference between them. When the experiment group containing gibberellin replaced the high water absorbent resin as its matrix, the root activity of Alfalfa had a significant increase and the combination of gibberellin and SAP made the root vitality to reach the maximum. The results showed that the addition of SAP could effectively improve the effect of gibberellin on the root activity of alfalfa. Maybe it's because SAP, as the substrate for plant growth, directly contacts the root, stimulates the root system to produce more root dehydrogenase and the root activity will increase with the increase of the content of the root dehydrogenase, so that the root's ability to absorb mineral nutrition and water will increase, thus providing better conditions for plant growth.

CONCLUSION

The eco-friendly SAP has no toxic effect on alfalfa and has an obvious promoting effect on its germination, root and seedling growth. At the same time, alfalfa is more beneficial to the growth of seedlings under the combined action of gibberellin and SAP. In addition, the excellent water retention effect of SAP increased the drought resistance of alfalfa seeds under drought conditions.

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