LRF-726
[1-8]

RESEARCH ARTICLE Legume Research- An International Journal

Cold Plasma Treatment for *Astragalus membranaceus* Seed Germination and Seedling Growth Improvement

Shaochong Wei[1], Lu Zhao[1,2], Zhen Liu[1], Xiaoyu Zhang[1], Yunting Hui[3], Decheng Wang[3], Zhongkuan Liu[4], Guixia Liu[1]

10.18805/LRF-726

ABSTRACT

Background: *Astragalus membranaceus* seeds have dormant properties that not only limit germination but also negatively affect the growth of seedlings. We investigated the enhancing effect of cold plasma treatment on seed germination and the positive effect on seedling growth of *Astragalus membranaceus* by treating its seeds with cold plasma at different power levels.

Method: The *Astragalus membranaceus* seeds were treated with cold plasma at power levels of 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 W for 15 seconds with helium gas and seeds without any treatment were used as control.

Results: The *Astragalus membranaceus* seeds were treated with cold plasma, which not only improved seed germination, but also had a positive effect on seedling growth, with the best results for the 80 W power cold plasma treatment. The 80 W power cold plasma treatment increased the germination rate, germination index and vigor index of *Astragalus membranaceus* seeds by 26.8%, 12.4% and 39.8%, respectively; increased root length, CAT activity and SOD activity of *Astragalus membranaceus* seedlings by 24.5%, 99.7% and 7.1%, respectively and decreased the relative conductivity and MDA content of *Astragalus membranaceus* seedlings by 6.6% and 17.6%, respectively; compared with that of the control group.

Key words: *Astragalus membranaceus*, Cold plasma, Seed germination, Seedling growth.

INTRODUCTION

Astragalus membranaceus (Fisch.) Bge. belongs to the family Leguminosae, is a perennial traditional Chinese herb and has important medicinal value (Liu *et al*., 2016; Li *et al*., 2017). The *A. membranaceus* seeds have dormant properties that can affect seed germination, thus limiting their cultivation and growth (Jia *et al*., 2012). Therefore, it is important to find suitable ways to break seed dormancy and improve the seedling growth of *A. membranaceus*.

Cold plasma (CP) is a pre-treatment technology used in agricultural sciences that can be used as an alternative to traditional pre-treatment methods such as physical scratching, heat treatment and chemical treatment (Feng *et al*., 2018). The CP technology plays an important role in optimizing crops by activating endogenous substances in seed rejuvenation, activating seed vitality, improving seed germination and stress resistance and improving crop quality and crop yield (Shao *et al*., 2015). The plasma treatment has been shown to be effective in improving seed germination and early growth in wheat, oat, oilseed rape, radish and alfalfa (Feng *et al*., 2018; Matra, 2016; Sera *et al*., 2017).

Current methods for breaking the dormancy of *A. membranaceus* seeds all have different drawbacks (Shao *et al*., 2012), so it is necessary to try more methods. In this study, a CP modified processor was used to treat *A. membranaceus* seeds at different power ratings. The objective of this study was to determine the promotion effect of CP treatment on *A. membranaceus* by analyzing seed germination and seedling growth after CP treatment of *A. membranaceus* seeds. This research could provide a theoretical basis for CP treatment technology in the utilization of *A. membranaceus* and determine the power of CP treatment applicable to *A. membranaceus* seeds, which would facilitate the cultivation and production of *A. membranaceus*.

[1]College of Life Science, Hebei University, Wusi Donglu 180, Baoding 071002, China.
[2]Hebei Research Institute of Microbiology Co. LTD, Wusi Zhonglu 2089, Baoding 071051, China.
[3]College of Engineering, China Agricultural University, Beijing 100083, China.
[4]Institute of Agro-resources and Environment, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang 050051, China.

Corresponding Author: Guixia Liu, College of Life Science, Hebei University, Wusi Donglu 180, Baoding 071002, China.
Email: liuguixia1971@163.com

How to cite this article: Wei, S., Zhao, L., Liu, Z., Zhang[1] X., Hui, Y., Wang, D., Liu, Z. and Liu, G. (2023). Cold Plasma Treatment for *Astragalus membranaceus* Seed Germination and Seedling Growth Improvement. Legume Research. doi: 10.18805/LRF-726.

Submitted: 24-11-2022 **Accepted:** 19-01-2023 **Online:** 21-02-2023

MATERIALS AND METHODS

Experimental materials

The *A. membranaceus* seeds were collected in August-September 2017 from the Baiyangdian region of Hebei province. The seed processing device was a CP seed processor developed by China Agricultural University. The device was sealed under a simulated vacuum state, *i.e*., after first creating the vacuum, an appropriate amount of helium gas was injected, which was then used as the medium for seed treatment (Fig 1).

Experimental design

On May 10, 2019, the *A. membranaceus* seeds were treated using a CP seed processor with treatment power levels set to 40, 60, 80, 100, 120, 140, 160, 180, 200, 220 and 240 W. The treatment time was 15 s and the gas was helium and the treated seeds were used in this experiment. In addition, *A. membranaceus* seeds without any treatment were used as a control group (CK) for this experiment.

The experiment was conducted on May 12, using the Petri dish filter paper method (two layers of filter paper in Petri dishes, moistened with deionized water). Fifty full, uniformly sized and undamaged *A. membranaceus* seeds treated with CP were selected and placed in Petri dishes, which were then incubated in an incubator at a relative humidity of 60%, constant temperature of 25°C and 12 hours of light, with four replicates of each treatment. The germination and growth were observed and recorded daily with appropriate supplemental water and the experiment was finished when the seeds did not germinate for three consecutive days.

Indicators and methods

Germination indicators

Germination rate =

$$\frac{\text{Number of germination at day 14 after treatment}}{\text{Total number of seeds}} \times 100\%$$

(Shang and Gao, 2018).

Germination index =

$$\sum \frac{\text{Number of seeds germinated on the same day}}{\text{Number of days of germination}}$$

((Shang and Gao, 2018).

Vigor index=

Average length of seedlings × Germination index (Li *et al*., 2014).

Seedling indicators

Shoot and root lengths

At the end of germination, 10 seedlings of *A. membranaceus* were randomly selected from each petri dish and measured with a ruler.

Catalase (CAT) activity

The ultraviolet spectrophotometry was used for the determination of CAT activity (Bao *et al*., 2017).

Superoxide (SOD) activity

The nitrogen blue tetrazolium photoreduction method was used for the determination of SOD activity (Bao *et al*., 2017).

Malondialdehyde (MDA) content

The thiobarbital method was used for the determination of MDA content (Bao *et al*., 2017).

Relative conductivity

The immersion method was used for the determination of relative conductivity (Hu *et al*., 2018).

Statistical analysis

The single factor random block analysis in SPSS 21.0 software was used for the statistical analysis of the experimental data and Duncan's post hoc test was used to make multiple comparisons of the experimental data (Yilmaz and Kulaz, 2019; Li and Luo, 2017). After the analysis is completed, the results obtained from the analysis were presented as mean ± standard error.

RESULTS AND DISCUSSION

Effects of CP treatments on seed germination of *A. membranaceus*

Germination rate

The 40W-220W CP treatment group increased the germination rate by 2.8%-26.8% than that of the control group (Fig 2). Further, the 80W CP treatment group had the highest germination rate of 60.0%, which was obviously increased by 26.8% than that of the control group (P<0.05).

Germination index

The CP treatment groups did not significantly affect the germination index compared to that of the control group (P<0.05) (Fig 3). Further, the 40W, 60W and 80W CP treatments elevated the germination index than that of the control group and the germination index peaked in the 80W CP treatment group with a 12.4% increase than that of the control group.

Vigor index

Compared to the control group, the CP groups treated with 40W-180W had an enhancing effect on the vigor index, able to increase by 1.1%-39.8%, with a peak in the 80W CP treatment group, which apparently increased by 39.8% than that of the control group (P<0.05) (Fig 4). However, the 200W, 220W and 240W CP treatment groups decreased the vigor index than that of the control group, with the 220W CP treatment group having the lowest vigor index, which was significantly lower than that of the control group. (P<0.05).

Effects of CP treatments on seedling growth of *A. membranaceus*

Shoot length

The 140W, 180W and 220W CP treatment groups had the same shoot length, which was obviously increased by 13.6% compared to that of the control group (P<0.05) (Fig 5). Moreover, the seedling lengths of 40W, 60W, 80W, 100W, 120W, 160W, 200W and 240W CP treatment groups were not significantly different than that of the control group (P<0.05).

Root length

The CP treatment groups enhanced the root length by 1.2%-36.8% than that of the control and the improvement reached 24.5%, 30.7%, 36.8% and 21.5% in the 80W, 100W, 140W and 180W CP treatment groups, respectively, with significant effects (P < 0.05) (Fig 6). Moreover, the root lengths of 40W, 60W, 120W, 160W, 200W and 240W CP treatment groups were not significantly different than that of the control group (P<0.05).

CAT activity

The 40W, 80W, 100W, 160W, 180W, 200W, 220W and 240W CP treatment groups showed a significant increase on the CAT activity than that of the control (P<0.05), while the 60W, 120W and 140W CP treatment groups did not significantly change the CAT activity than that of the control (P<0.05) (Fig 7). Further, the 200W CP treatment group had the highest CAT activity, with a significant increase of 301.0% over that of the control group (P<0.05).

SOD activity

The 60W, 80W, 100W, 160W and 240W CP treatment groups showed a significant increase on the SOD activity than that of the control (P<0.05), although there were no significant differences between the SOD activities of the 60W, 80W, 100W, 160W and 240W CP treatment groups (P<0.05) (Fig 8). The SOD activity was significantly reduced in the 120W and 140W CP treatment groups compared to the control group and was lowest in the 120W CP treatment group (P<0.05).

Relative conductivity

Compared to the relative conductivity of the control group, each CP treatment group was significantly reduced by 5.9%-

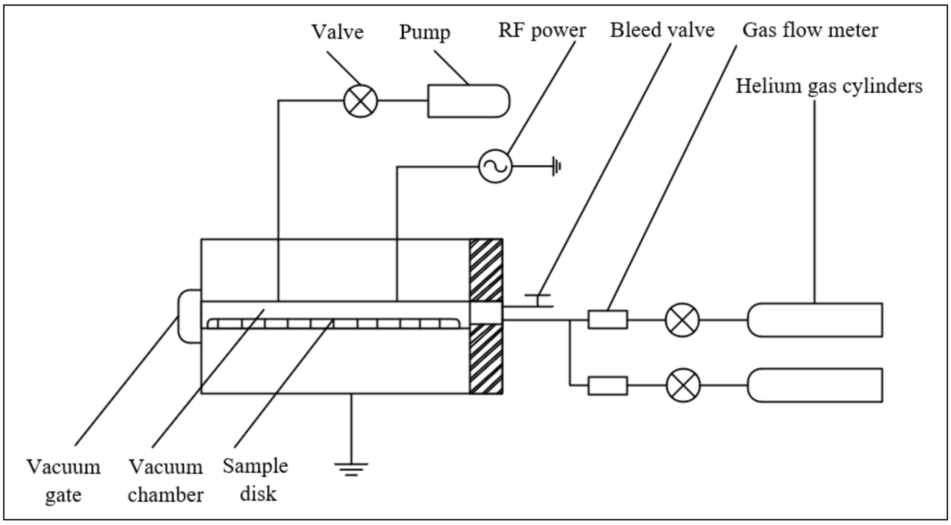

Fig 1: Schematic of low temperature plasma test platform.

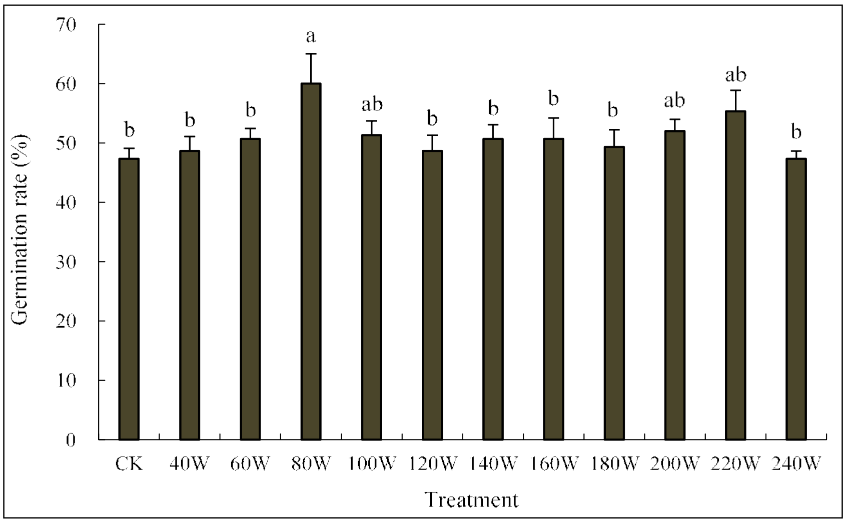

Fig 2: Effects of the CP treatments on the germination rate of *Astragalus membranaceus* seeds.

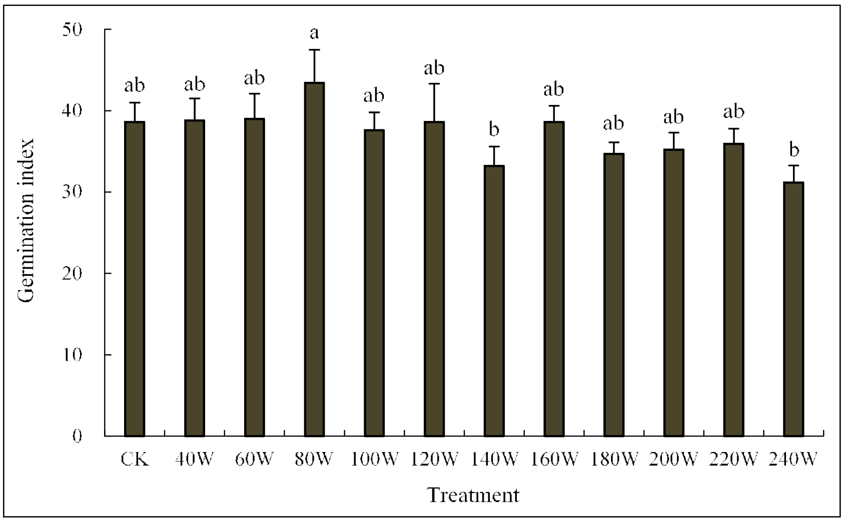

Fig 3: Effects of the CP treatments on the germination index of *Astragalus membranaceus* seeds.

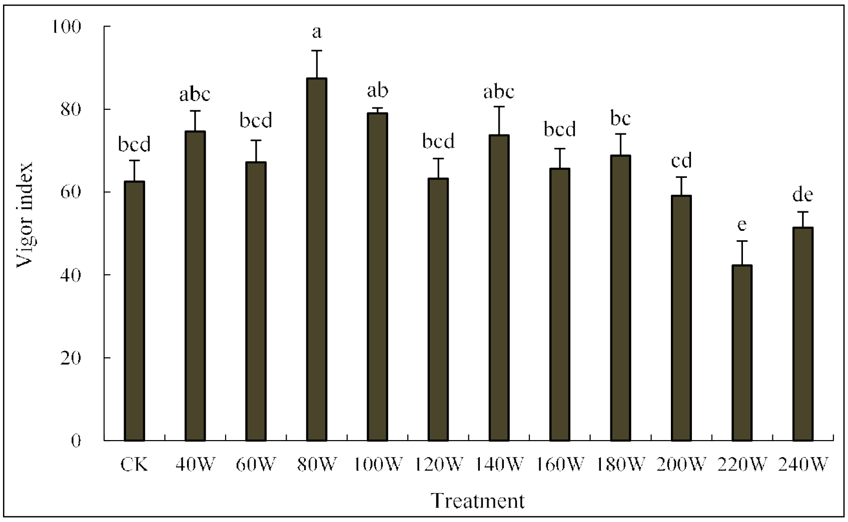

Fig 4: Effects of the CP treatments on the vigor index of *Astragalus membranaceus* seeds.

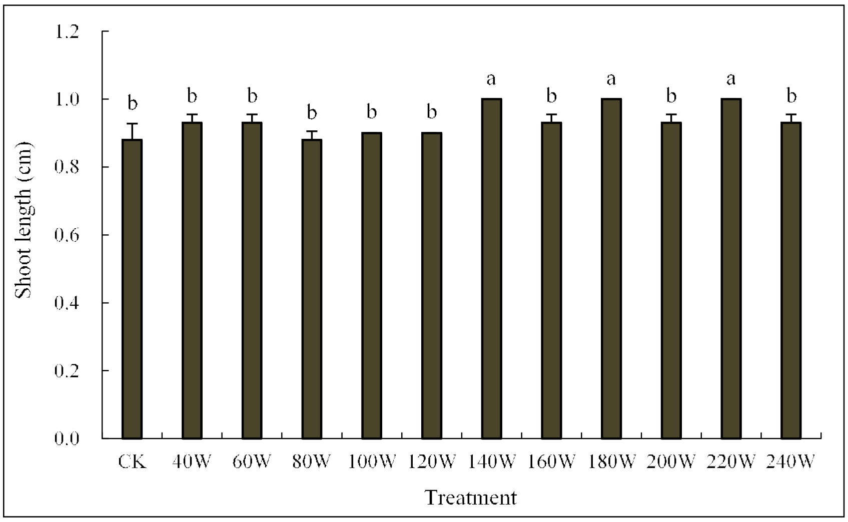

Fig 5: Effects of the CP treatments on the shoot length of *Astragalus membranaceus* seedlings.

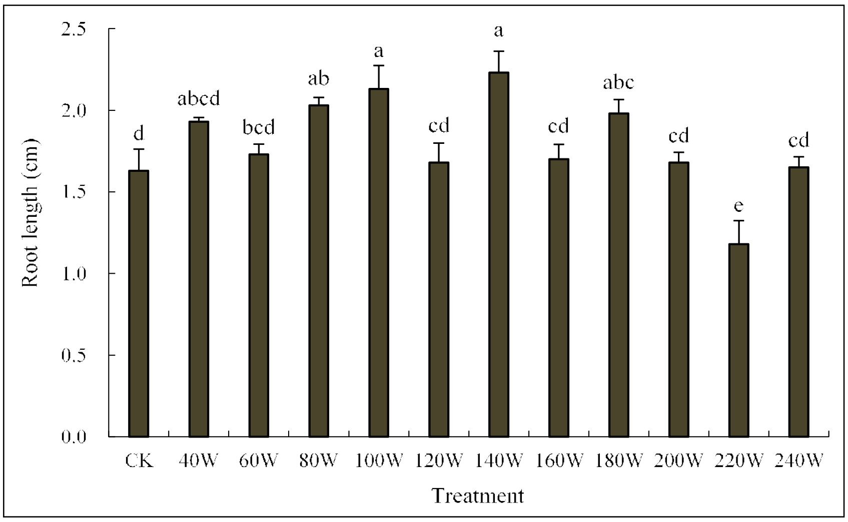

Fig 6: Effects of the CP treatments on the root length of *Astragalus membranaceus* seedlings.

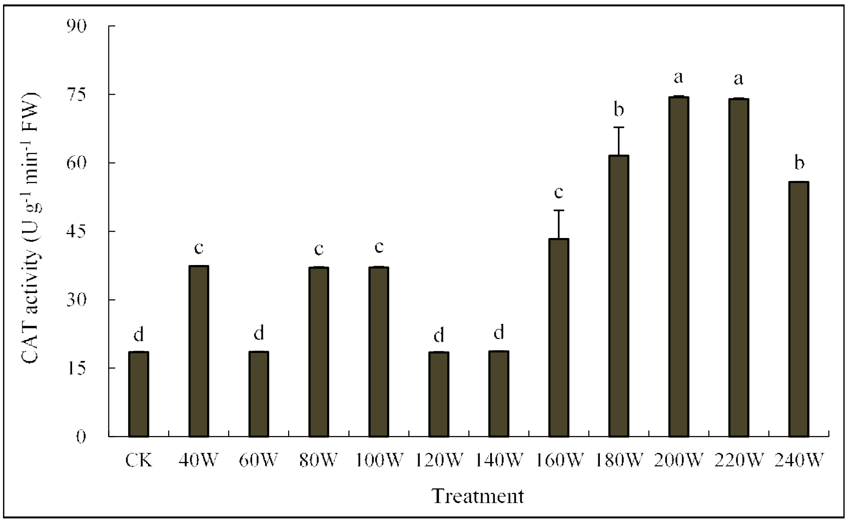

Fig 7: Effects of the CP treatments on the CAT activity of *Astragalus membranaceus* seedlings.

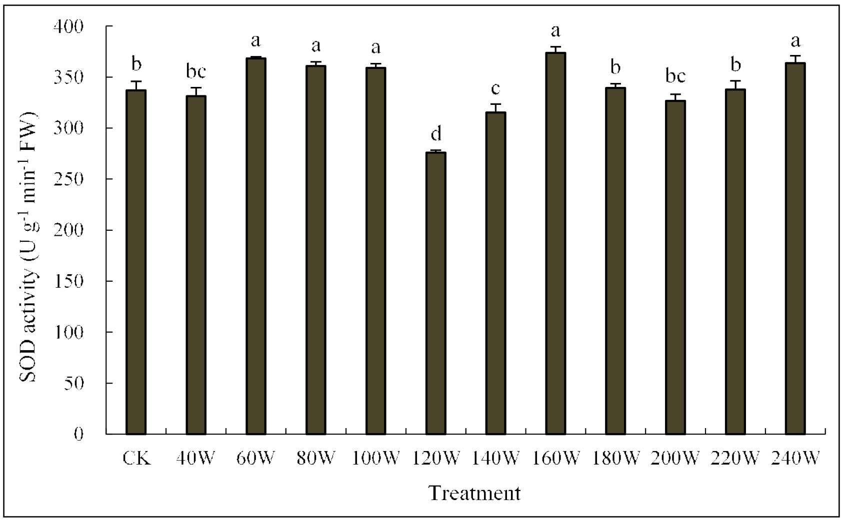

Fig 8: Effects of the CP treatments on the SOD activity of *Astragalus membranaceus* seedlings.

11.3% ($P<0.05$) (Fig 9). Further, the 200W CP treatment group had the lowest relative conductivity, which reduced by 11.3% compared to that of the control group.

MDA content

Compared to the control group, the 40W, 60W, 80W, 100W, 120W, 140W and 200W CP treatment groups significantly reduced the MDA content by 9.1%-24.8% ($P<0.05$) (Fig 10). Further, the effect of 160W and 180W CP treatment groups on MDA content was not significant compared to that of the control, although they were 7.5% and 6.8% higher, respectively ($P<0.05$).

Effects of CP treatments on seed germination of *A. membranaceus*

In this study, the *A. membranaceus* seeds treated with CP increased germination rate by 2.8%-26.8%, germination index by 0.5%-12.4% and vigor index by 1.1%-39.8%, indicating that CP treatment could promote seed germination, similar to the study by Li *et al.* (2014). This may be due to the ability of CP to increase the permeability of the plasma membrane by altering the structure of the seed coat during treatment of the seeds, thus enhancing the material and energy cycling inside and outside the seed coat (Feng *et al.*, 2018). Additionally, ultraviolet irradiation and active free radicals may also have a facilitating effect on seed germination by etching the seed coat after plasma treatment (Feng *et al.*, 2018; Wu *et al.*, 2007).

Effects of CP treatments on seedling growth of *A. membranaceus*

After the *A. membranaceus* seeds were treated with CP, the shoot length increased by 2.3%-13.6% and the root length increased by 1.2%–36.8%, indicating that CP treatment with appropriate power can promote the growth of shoot length and root length of the *A. membranaceus* seedlings, which was similar to the results of Li *et al.* (2015).

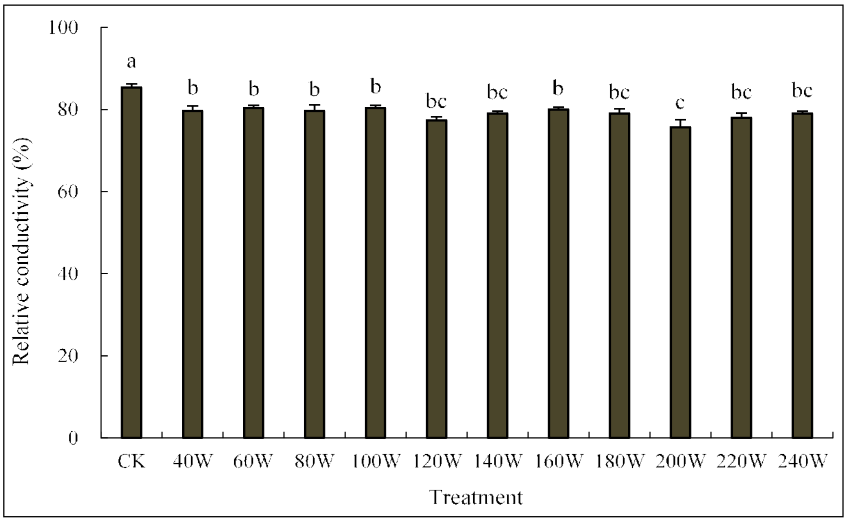

Fig 9: Effects of the CP treatments on the relative conductivity of *Astragalus membranaceus* seedlings.

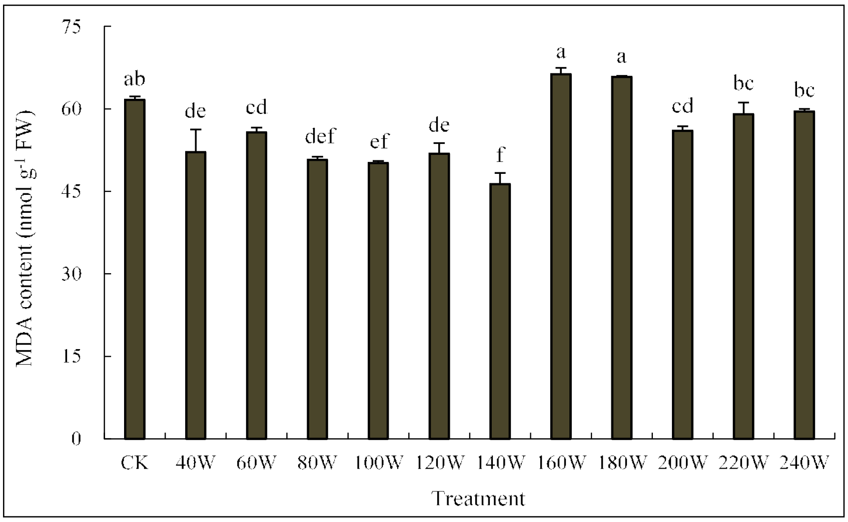

Fig 10: Effects of the CP treatments on the MDA content of *Astragalus membranaceus* seedlings.

After the *A. membranaceus* seeds were treated with CP, the CAT activity and SOD activity of *A. membranaceus* seedlings increased by 0.1%-301.0% and 0.3%-10.9%, respectively, indicating that CP treatment of *A. membranaceus* seeds was able to positively affect SOD and CAT activities of seedlings during growth. Previous studies have confirmed that CP treatment can improve the activities of CAT and SOD in *Poa pratensis* and *Arabidopsis thaliana* seedlings (Zhang *et al*., 2022; Wang *et al*., 2020) and the results of the present study were similar to their results.

This study showed that CP treatment reduced the relative conductivity and MDA content of *A. membranaceus* seedlings. After the *A. membranaceus* seeds were treated with CP, the relative conductivity of seedlings was reduced by 5.9%-11.3% and the reduction in relative conductivity helped to protect the integrity of cell membranes during stress, which was was similar to the results of Wu *et al*. (2007). After the *A. membranaceus* seeds were treated with CP, the MDA content was reduced by 3.4%-24.8% and the low level of MDA could reduce the degree of plant tissue injury, which was similar to the results of Ma *et al*. (2020).

CONCLUSION

The seed germination and seedling growth of *A. membranaceus* can be promoted after *A. membranaceus* seeds were treated with CP treatment. In this experiment, the best performance of the 80W CP treatment group can be found in combination with combining seed germination, seedling growth and power consumption, which indicates that 80 W power was the most suitable power for CP treatment of *A. membranaceus* seeds.

The *A. membranaceus* seeds treated with cold plasma improved seed germination and seedling growth of *A. membranaceus* and the cold plasma treatment with 80 W power was the most suitable treatment for *A. membranaceus seeds*.

The *A. membranaceus* seeds treated with 80 W cold plasma increased germination rate by 26.8%, germination index by 12.4% and vigor index by 39.8%, compared to the control.

The *A. membranaceus* seeds treated with 80 W cold plasma also effectively improved seedling growth and root length, SOD activity and CAT activity of *A. membranaceus* seedlings were 24.5%, 99.7% and 7.1% higher than the control, respectively and relative conductivity and MDA content of *A. membranaceus* seedlings were 6.6% and 17.6% lower than the control, respectively.

ACKNOWLEDGEMENT

This work was supported by the Hebei Grass Industry Innovation team of the Modern Agricultural Industry Technology System (HBCT2018050204) and Key Research and Development Project of Hebei Province (20327508D).

Conflict of interest: None.

REFERENCES

Bao, X.X., Bao, X.P., Lian, Y. (2017). Effects of Drought Stress on Physiological and Biochemical Parameters of *Allium polyrhizum* in Inner Mongolia. Acta Agriculturae Boreali-Sinica. 32(01): 233-238.

Feng, J.K., Wang, D.C., Shao, C.Y., Zhang, L.L., Tang, X. (2018). Effects of cold plasma treatment on alfalfa seed growth under simulated drought stress. Plasma Science and Technology. 20(03): 33-139.

Hu, C.Y., Cao, Q., Han, Z.J., Zhou, D.D. (2018). Response of *Halostachys capsica* Seeds and Seedlins to $NaHCO_3$ Stress. Seed. 37(04): 40-44.

Jia, L.H., Fang, S.C., Li, A.P. (2012). Study on Seed Dormancy and Dormancy Release Methods of *Astragalus membranaceus* (Fisch) Bge. Horticulture and Seed. 12: 43-45+62+2.

Li, C.H., Dong, Y.L., Li, Z.H., Zhang, R.H., Qin, R.L., Wang, L., Shi, W.Y. (2017). Effects of traditional Chinese herbal medicines on egg production, egg quality and antioxidant enzyme activities in aged laying hens. Indian Journal of Animal Research. 51(2): 327-331.

Li, J. and Luo, Y.W. (2017). Soluble sugars and myo-inositol phosphates during germination and seedling growth of green and white faba bean (*Vicia faba* L.). Legume Research. 40(1): 47-54.

Li, L., Jiang, J.F., Li, J.G., Shen, M.C., He, X., Shao, H.L., Dong, Y.H. (2014). Effects of cold plasma treatment on seed germination and seedling growth of soybean. Scientific Reports. 4(1): 1-7.

Li, L., Shen, M.C., Li, J.G., Dong, Y.H. (2015). Effects of treating seeds with cold plasma on seed germination and seedling growth of oil crops. Acta Agriculturae Jiangxi. 27(08): 1-5.

Liu, X., Duan, L., Chang, Z.Y. (2016). A cladistic analysis of medical *Astragalus penduliflorus* Lam. complex (Leguminosae: Papilionoideae) in China and its taxonomic implications. Legume Research. 39(5): 698-703.

Ma, Y.T., Mi, M., Bai, M.M., Ma, H.L., Liu, X.N., Yu, J.H., Shao, H.L. (2020). Effects of CPT on Seed Germination and Seedling MDA Content of Alfalfa. Journal of Anhui Agricultural Sciences. 48(08): 1-5+10.

Matra, K. (2016). Non-thermal plasma for germination enhancement of radish seeds. Procedia Computer Science. 86: 132-135.

Sera, B., Sery, M., Gavril, B., Gajdova, I. (2017). Seed germination and early growth responses to seed pre-treatment by non-thermal plasma in hemp cultivars (*Cannabis sativa* L.). Plasma Chemistry and Plasma Processing. 37(1): 207-221.

Shang, H.Q. and Gao, C.Y. (2018). Effect of PEG Seed Soaking on Seed Germination and Bud Growth of Pepper. Seed. 37(10): 102-106.

Shao, C.Y., Wang, D.C., Tang, X., Zhao, L.J. (2012). Application status and development trend of arc light magnetized plasma seed treatment equipment. China Seed Industry. 08: 1-3.

Shao, C.Y., Wang, D.C., Yang, P., Liu, L. D., You, Y., Wang, G.H., Tang, X., Liang, F.C., Zhang, L.L. (2015). Epigenetic study of alfalfa based on cold plasma seed treatment technology. China Seed Industry. 03: 12-14.

Wang, J.Q., Cui, D.J., Yin, Y., Li, Y.L., Jiao, Z. (2020). Effect of cold atmospheric pressure plasmas treatment on antioxidant system in *Arabidopsis thaliana* seedlings. Plant Physiology Journal. 56(03): 423-430.

Wu, Z.H., Chi, L.H., Bian, S.F., Xu, K.Z. (2007). Effects of plasma treatment on maize seeding resistance. Journal of Maize Sciences. 05: 111-113.

Yilmaz, H. and Kulaz, H. (2019). The effects of plant growth promoting rhizobacteria on antioxidant activity in chickpea (*Cicer arietinum* L.) under salt stress. Legume Research. 42(1): 72-76.

Zhang, Y.J., Guo, R., Zhang Z.H., Zhang, Y., Ma, H.L., Dong, W.K. (2022). Effect of Clod Plasma Treatment on Salt Resistance of *Poa pratensis* Seedlings. Acta Agrestia Sinica. 30(09): 2365-2374.