J. Farm Sci., 36(2): (206-208) 2023

RESEARCH PAPER

Effect of cold plasma on seed quality in soybean (*Glycine max* L.Merril)

GAGAN A V[1], DESHPANDE V K[1], VINOD KUMAR[2], RAVIKUMAR R HOSAMANI[3]

[1]Department of Seed Science and Technology, [3]Department of Biotechnology
[2]Regional Research Station, ICAR-IGFRI, Dharwad
College of Agriculture,University of Agricultural Sciences, Dharwad - 580 005, India
E-mail: gaganav250898@gmail.com

(Received: April, 2023 ; Accepted: May, 2023)

Abstract: Soybean is a major oilseed crop rich in oil and protein content which is a part of diet in many countries. Soybean seeds have thin and fragile testa, hence susceptible to mechanical damage resulting in poor and non-uniform germination which subsequently leads to poor field establishment and yield reduction. A laboratory experiment was carried out in the Department of Seed Science and Technology and Department of Biotechnology, University of Agricultural Sciences, Dharwad during 2021-22. The experiment was laid out under CRD comprising eight treatments in four replicates, where medium vigour soybean seeds of 68 per cent initial germination were subjected to assess the effect of cold plasma treatments (0, 3, 5, 7, 10, 13, 16 and 19 sec) on seed quality parameters at 200 KHz frequency, 980 watt power by using nitrogen gas at Indian Institute of Space Science and Technology, (IIST) Thiruvananthapuram, Results revealed that the effect of cold plasma on seed quality parameters of soybean varied with different durations. Enhancement in seed germination and vigour index was observed at 3 seconds (72.00%) and 5 seconds (70.00%) which exposure recorded 5.88 per cent and ~3 per cent higher germination over control. Further higher duration of treatment (10 to 19 seconds) deteriorated or had significantly negative impact on seed germination, seedling vigour which was evident through decrease in shoot length, root length, seedling dry weight, total dehydrogenase enzyme activity correspondingly. The present study showed that cold plasma had an active effect on seed quality parameters at lower dose. Three seconds (T2) cold plasma noticed highest stimulatory effect among the different treatments and next best was five seconds (T3).

Key words: Dehydrogenase activity, Plasma treatment, Soybean, Speed of germination,Vigour index

Introduction

Soybean is a major worldwide oilseed crop rich in oil and protein content which is a part of diet in many countries. Soybean seeds have thin and fragile testa, hence susceptible to mechanical damage resulting in poor and non-uniform germination which subsequently leads to poor field establishment and yield reduction. Promoting seed germination and vigour is the most direct way to improve soybean production which is achieved by treating the seeds with cold plasma, a fast, economic and pollution free method to improve seed performance, seedling growth and crop yield.Cold Plasma or non-thermal plasma is one of the fourth fundamental states of matter, the others being solid, liquid and gas. Cold plasma is an ionized gas (constitute of proton, neutron, electron and charged atom or molecule), where the thermal motion of the ions can be ignored. Can be created by heating a gas or subjecting it to a strong electromagnetic field which behaves differently from a gas. In present days cold plasma has its wider application in medical field (to sterilize the surgical instruments), in context of food processing industry, in-packaging of meat which found as a novel non-thermal technology for microbial inactivation and shelf-life extension and also as emerging trend in seed treatment (physical treatment) for enhancing seed quality and inactivation of microbes on seed surface (Seluk *et al*. 2008). Hence the present study was under taken at UAS, Dharwad, Karnataka, India.

Material and methods

The study was under taken at Department of Seed Science and Technology, UAS, Dharwad, Karnataka, India on soybean variety JS-335 with 8 treatments and 4 replications using CRD design

Treatment details: Medium vigour soybean seeds of 68% initial germination were subjected to assess the effect of cold plasma treatments (0, 3, 5, 7, 10, 13, 16 and 19 sec), at 200 KHz frequency, 980 watt coupled power by using nitrogen gas at Indian Institute of Space Science and Technology, IIST, Thiruvananthapuram, Kerala.) and mentioned below various seed quality parameters were analyzed.

Observations recorded

Seed germination (%)

Germination test was conducted in four replications each of 100 seeds as per ISTA procedure (Anon., 2019) by adopting paper towel method. The rolled paper towels were placed at slanting position in a seed germinator at temperature 25±1 and 95±1 per cent relative humidity. Seedlings were counted on fifth day as first count and 8th day as final count and expressed in percentage.

Root length (cm)

Ten normal seedlings were selected randomly from each treatment of the germination test on 8th day was used for measuring root length. The root length was measured from the tip of primary root to the base of hypocotyl and the mean root length was expressed in centimeters.

Shoot length (cm)

Ten normal seedlings were selected randomly from each treatment of the germination test on 8th day was used for measuring shoot length. The shoot length was measured from the base of the primary leaf to the base of hypocotyl and the mean shoot length was expressed in centimeters.

Seedling dry weight (g/10 seedlings)

The normal ten seedlings used for measuring root and shoot length were kept in butter paper bag and dried in hot air oven maintained at 75^{0}C temperature for 24 hours. Then the seedlings were weighed after allowing them to cool at ambient temperature in a desiccator for 60 minutes and expressed in milligrams per ten seedlings.

Seedling vigour indices

The seedling vigour index was computed by adopting the method suggested by Abdul-Baki A A and Anderson (1973) and expressed as an index number.

Seedling vigour index I = Germination % × [Shoot length (cm) + Root length (cm)].

Seedling vigour index II = Germination % × Seedling dry weight (mg/10 seedlings).

Speed of germination

The seeds showing radicle protrusion were counted every day up to final count. The speed of germination was calculated by using the formula given by Maguire (1962) and the results were expressed in numbers.

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots + \frac{X_n - X_{(n-1)}}{Y_n}$$

Where, X_n = Number of seeds germinated at nth count

Y_n = Number of days from sowing to nth count

Total dehydrogenase activity

Dehydrogenase activity was determined by using the tetrazolium (TTC) staining method. 25 representative seeds from each treatment in four replications were taken and preconditioned by soaking in water overnight at room temperature. Embryo was excised from the seeds and was steeped in 0.5 per cent solution of 2, 3, 5- triphenyltetrazolium chloride and kept in dark for two hours at 40 °C for staining.

The stained seeds were thoroughly washed with water and then soaked in 10 ml of 2 methoxy ethanol (methyl cellosolve) and kept overnight for its change to red colour formazan. The intensity of red colour was measured using UV- VIS spectrophotometer using blue filter at 470 nm wave length and methoxy ethanol was used as blank. The OD value obtained was recorded as the dehydrogenase enzyme activity (Kittoch and Law, 1968).

Statistical analysis

Fisher and Yates (1963) method of variance was applied for the analysis and interpretation of experimental data. The level of significance in the 'F' test and t test was at 1 per cent, and critical difference was calculated whenever the 'F' test was found significant after comparing with the treatments.

Results and discussion

The present study showed that cold plasma had an active effect on seed quality parameters at lower dose. Three seconds (T_2) cold plasma treatment produced the highest stimulatory effect among the different treatments (Table 1) along with increase in the enzymatic activity Fig 2 and next

Table 1. Effect of cold plasma treatment on germination, root length, shoot length, seedling dry weight and seedling vigour index-I of soybean cv. JS-335

Treatments (T)	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling dry wight (mg/10 seedlings)	Seedling vigour index-I	Speed of germination	Total dehydrogenase activity @ 470 nm
T_1- control	68.00 (55.56)	15.38	16.23	772.5	2224 [3.32]	5.48	0.763
T_2- 3 seconds	72.00 (58.05)	17.18	17.46	875.3**	2511 [3.40]	6.86**	0.989**
T_3- 5 seconds	70.00 (56.79)	16.05	16.84	796.0	2449 [3.36]	6.17	0.895
T_4- 7 seconds	65.67 (54.13)	14.82	16.26	742.1	2364 [3.31]	4.31	0.803
T_5- 10 seconds	62.00 (51.94)	14.16	16.09	741.1	2069 [3.23]	3.83	0.702
T_6- 13 seconds	58.00 (49.60)	13.97	15.59	716.1	1860 [3.15]	3.33	0.700
T_7- 16 seconds	54.67 (47.70)	13.26	15.18	696.3	1683 [3.10]	2.82	0.660
T_8- 19 seconds	53.00 (48.45)	12.56	14.75	696.3	1608 [3.07]	2.00	0.639
S.Em .±	1.26	0.37	0.40	18.59	0.04	0.106	0.019
C.D. @ 0.01	5.18	1.53	1.69	76.79	0.149	0.437	0.079
CV (%)	4.13	4.38	4.28	4.26	1.93	4.22	4.35

( ) Numbers in the parenthesis indicate arc sine transformed values

[ ] Numbers in the parenthesis indicate log transformed values

** Significant at 1 per cent

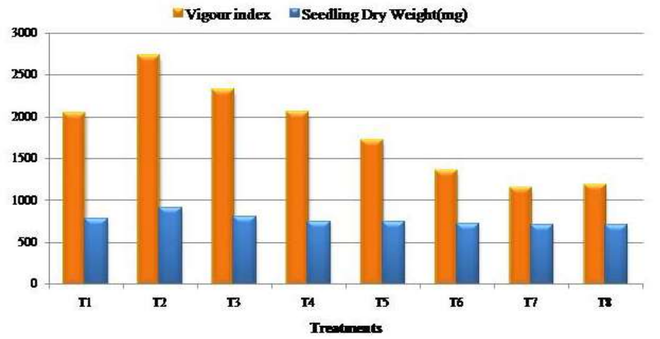

Fig1. Effect of cold plasma on vigour index and seedling dry weight (mg/10 sedlings) of Soybean (JS-335)

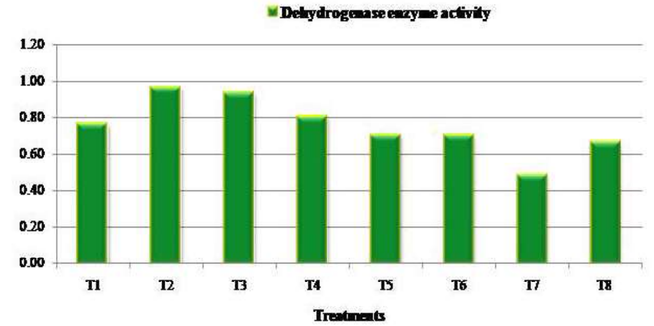

Fig 2. Effect of cold plasma on total Dehydrogenase activity at 470 nm

best was five seconds (T_3). Cold plasma treatment with higher energy and long duration had deleterious influence on seed quality parameter especially germination (T_8) due to more seed damage during the treatment. The results are in conformity with Ling *et al*.(2016). Seed immersed into cold plasma are subjected to an attack by free radicals and are bombarded by ions resulting in seed coat erosion and creates etched surface. The altered seed coat surface could increase the hydrophilic ability of the seed, and eventually improve the water uptake of the seeds which is necessary for seed germination (Bormashenko *et al*. 2012, Wang *et al*. 2017, Mitra *et al*. 2014). By increasing in seed surface hydrophobicity, it reduces the water contact angle from 115° to 0° which in turn increases water uptake and triggering further metabolic reactions (Bormashenko *et al*. 2012). According to Ling *et al*. (2014) cold plasma treatment reduces the contact angle of soybean seeds from 70.14° to 20.94°. The plasma-induced changes in the physicochemical characteristics of the seed surface and the improvements in water absorption are well supported by sufficient data as clearly represented in the Table 1 and graphically depicted in Fig 1 and 2.

Conclusion

Cold plasma treatments have the potential to enhance the seed germination, seedling growth, root length, shoot length and seedling dry weight of soybean. The improvement in these seed quality parameters of soybean in response to cold plasma treatment appears to be a consequences of the increase in seed coat permeability, water uptake, seed reserve utilization and protein content. This treatment may have positive effect on soybean seed quality parameters and more studies are needed to investigate the influence of cold plasma on soybean as well as to elucidate the mechanism that promote the effect of cold plasma treatment on seed quality parameters.

References

Abdul-Baki A A and Anderson J D, 1973, Vigour determination in soybean by multiple criteria. *Crop Science*, 13: 630-633.

Anonymous, 2019, ISTA-International Rules for Seed Testing. *Proceedings of International Seed Testing Association*, 31: 1-152.

Bormashenko E, Grynyov R, Bormashenko Y and Drori E, 2012, Cold radio frequency plasma treatment modifies wettability and germination speed of plant seeds. *Scientific Reports,* 2(1): 1-8.

Fisher R A and Yates F, 1963, Statistical tables for biological, agricultural and medical research. Edinburgh; *Oliver and Boyd Publishers*, London (England)

Ling L, Jangang L, Minchong S, Junfeng H, Chunlei Z and Yuanhua D, 2016, Cold plasma treatment enhances oilseed rapseed germination under drought stress. *Scientific Reports*, 5:1033.

Ling L, Jangang L, Minchong S, Junfeng H, Hanhang S, Yuanhua D and Jiafeng J, 2016, Improving seed germination and peanut yields by cold plasma treatment. *Plasma Science and Technology*, 18(10): 1027-1033.

Ling L, Jiafeng J, Jiangang L, Minchong S, Xin H, Hanliang S and Yuanhus D, 2014, Effects of cold plasma treatment on seed germination and seedling growth of soybean. *Science Reports*, 4(1): 1-7.

Maguire J D, 1962, Speed of germination in selection and evaluation for seedling vigour. *Crop Science*, 2: 176-177.

Mitra A, Li, Y F, Klampfl T G, Shimizu T, Jeon J, Morfill G E and Zimmermann J L, 2014, Inactivation of surface-borne microorganisms and increased germination of seed specimen by cold atmospheric plasma. *Food and Bioprocess Technology,* 7(3): 645-653.

Seluk M, Oksuz L and Basaran P, 2008, Decontamination of grains and legumes infected with *Aspergillus* spp. and *Penicillum* spp. by cold plasma treatment. *Bioresource Technology,* 99(11): 5104-5109.

Wang S, Park Y S, Yang Y, Borrego E J, Isakeit T, Gao X and Kolomiets M V, 2017, Seed derived ethylene facilitates colonization but not aflatoxin production by *Aspergillus flavus* in maize. *Frontiers in Plant Science,* 8: 415.

Kittoch D L and Law A G, 1968, Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germination of wheat seeds. *Agronomy Journal*, 60:286-288.