Impact of cold plasma irradiation on the germination indices in green gram *(Vigna radiata)* under water stress

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Abstract: The impact of cold plasma treatment on the germination characteristics in green gram under drought stress induced by Poly ethylene glycol PEG-6000 (10% w/v) was investigated. The samples were treated with inductively coupled radiofrequency (RF) plasma of 13.56 MHz. at different power levels of plasma discharge 30W, 50W and 70W for different time intervals by varying the feed gas O_2 and N_2. The plasma treated seed samples were subjected to germination assay under water stress simulated by 10% PEG. In the germination assay, it was observed that the germination potential, germination rate, germination index in water stressed sample was reduced by 16.66 %, 13.34 %, 15.93 % respectively when compared with water unstressed control whereas cold plasma treatments significantly increased the germination potential by 20%, germination rate by 16.67% and germination index by 24.21 % when compared to water stressed sample. Among the treatments, the germination potential and germination rate was higher for the N_2 plasma irradiation at 30W for 10 minutes, the germination index was maximum (29.5) for N_2 plasma irradiation at 50 W for 20 minutes and the vigour index was maximum (763.56) for N_2 plasma irradiation at 50 W for 10 minute exposure. The impact of cold plasma treatment was more intense on root growth than shoot growth under water stress. Cold plasma treatment could be used in an ameliorative way to improve germination and protect seedlings against damage caused by drought stress.

Index Terms - Cold Plasma – Green gram - water stress – Germination characteristics

I. INTRODUCTION

Drought, salinity and seed dormancy are the major problems that affect germination of seeds, crop growth and yield across the world. Water limitation is undoubtedly a critical environmental constraint hampering crop production in arid and semiarid areas. Sustaining the quality of seeds is essential to supply nutrition to the growing world population. The most commonly used methods to enhance seed germination are the chemical methods which include agrochemicals, fungicides, insecticides and hormones that leave harmful residues. Cold plasma seed treatment is a novel emerging eco-agricultural technology for improving seed performance, plant growth and productivity and enhances the ability of plants to cope with biotic and abiotic stress. It can significantly benefit the seed germination during drought conditions. It is based on low-level radiation which can activate the vitality of seeds but without causing gene mutations, and hence different from mutation breeding by particle beam. Plasma, often referred to as the fourth state of matter, is a quasi-neutral ionized gas, containing an array of active species of electrons, photons, free radicals and ions exhibiting unique properties. (Ekezie, F.C *et al*., 2017 and Dasan *et al*., 2017). Reactive species formed in the plasma has the ability to breakdown seed dormancy and increase the seed germination rate. The seed germination rate can be increased on application of cold plasma by both direct and indirect treatments. In direct treatment method the seeds are directly placed in between the electrodes or placed under the plasma regime. Recently, the indirect treatment through the application of plasma activated water (PAW) has gained importance. Cold plasma treatment is thus a fast, economic and pollution-free method to improve seed performance and crop yield. It has essential roles in a broad spectrum of developmental and physiological processes in plants, including reducing the bacterial bearing rate of seeds, changing seed coat structures, increasing the permeability of seed coats. This phenomenon has been demonstrated in several plants such as *Chenopodium album, Oryza sativa, Triticum aestivum, Lycopersicon esculentum and Solanum melongena L.*. The synergistic effect of cold plasma can replace the traditional seed disinfection solutions and chemical seed germination enhancers (Thirumdas *et al*., 2017).

Green gram is the most important legume as it is an excellent source of Protein and essential minerals. It has high nutritive value and the seed contains 24.20% protein content, 1.30% fat, and 60.4% carbohydrates; 118 mg calcium (Ca) and 340 mg phosphorus (P) per 100g respectively (Imran *et al*., 2015). It is an important economic crop in South East Asia. Diseases and abiotic stresses, such as drought, heat, water logging and salinity, can lead to a considerable loss in nutritional quality and economic yield of mung bean. Drought stress in green gram usually induces the accumulation of reactive oxygen species (ROS), such as superoxide radical, hydrogen peroxide, superoxide and singlet oxygen, which can destroy normal metabolism through oxidative damage to lipids, proteins and nucleic acids. On the other hand, antioxidant enzymes including superoxidase, peroxidase and catalase become activated to combat ROS. Osmotic adjustment is another important strategy to deal with drought stress. It has been observed that plants accumulate osmolytes such as soluble sugars and soluble proteins to maintain osmotic equilibrium and the integrity of membranes when they are subjected to drought stress. Though the effect of cold plasma irradiation on germination characteristics in green gram has been investigated, evaluation of cold plasma effect under drought stress is least observed. The objective of the current study was to investigate the influences of cold plasma treatment on seed germination and seedling growth in green gram under drought stress simulated by PEG- 6000.

II. Materials and Methods

2.1. Plant material:

Seeds of green gram used for the analysis were procured from the local market.

2.2. Cold Plasma Treatment:

Healthy uniform seeds selected were subjected to Cold plasma treatment. The core of the cold plasma processing system is the cold plasma generator Gauss Magnetics ICP - 100. Seeds were exposed to an inductively coupled plasma discharge under the following parameters: the radio frequency was on the order of 13.56 MHz. The pressure was 150 Pa and the volume of the discharge chamber was 1200 mm * 180 mm * 20 mm. The source of feed gas utilized for the study was O_2 and N_2. The power of discharge was varied at three different levels 30W, 50W and 70 W. The time span of irradiation was varied from 10 minutes to 30 minutes. Meanwhile, plasma untreated seeds served as control.

2.3. Seed Germination Assay:

The experiment was carried out in completely randomized design with three replications. PEG 6000 (Polyethylene glycol) (10% W/V) was used for simulating water stress. The seed germination tests were carried out. Seeds from each of the different plasma treatments obtained by varying N_2 and O_2 feed gas and varying voltage at 30W, 50W and 70 W for a period of 10 min, 20 min and 30 min were placed on filter paper in Petri dishes moistened with 10 ml of PEG solution and a set of plasma untreated seeds were placed on a petri dish in filter paper moistened with 10 ml of distilled water (water unstressed control) and another set placed in 10 ml of PEG solution (water stressed). Seeds were considered to be germinated when the radicals were half the seed length and when both the plumule and radicle were extended to more than 2 mm. Germination percentage was recorded every 24 h for 7 days. Germination potential (%); Germination Rate (%); Germination index and Vigour Index were determined following the procedure of Sadeghi *et al.* (2011) as follows:

Germination potential (%) = Number of seeds germinated in 3 d/total number of seeds x 100 %
Germination rate (%) = Number of seeds germinated in 7 d /total number of seeds x 100 %
Germination index Gi = $\sum Gt/Dt$
where Gt = number of germinated seeds on the t day; Dt - germination days.
Vigor index = Germination index x (Shoot + Root) Length

2.4. Seedling growth characteristics:

Root length, shoot length, dry weight of roots and shoot were recorded. Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transmission zone up to the base of the cotyledons. The root and shoot length was measured with the help of a thread and scale. The dry weight of root and shoot of seedlings were determined.

2.5. Statistical Analysis:

The data were subjected to one-way analysis of variance (ANOVA), and treatment means separated from the control at $P < 0.05$ or 0.01. Statistical analysis was done using AGRES software packages. All data are presented as the mean value ± standard error (SE) of three replicates.

III.RESULTS AND DISCUSSIONS

3.1. Germination characteristics:

Germination potential, germination index are the most significant parameters of biological vigor of the seed. Positive influence of cold plasma treatment was recorded on seed germination under drought stress in green gram which is evident from Table 1 and Fig 1-4. Germination improvement was significantly more pronounced in plasma treated samples than water stressed sample. The germination potential, germination rate, germination index and vigor index in water stressed treatment was significantly reduced by 16.66 %, 13.34 %, 15.93 % and 50.4% respectively when compared with control (water unstressed). However, on cold plasma treatment there was marked enhancement in germination parameters and there was increase in germination potential by 20% , germination rate by 16.67% , germination index by 24.21 % respectively, compared to the water stressed sample. It was observed that among the treatments, the maximum germination potential and germination rate was observed in both N_2 plasma and O_2 plasma at 30W for 10 minutes treatment whereas germination index was higher (29.5) in the N_2 plasma at 50 W for 20 minutes and in O_2 plasma, 30W for 10 minute treatment recorded higher Germination index of 29.25. Similarly, the vigour index was maximum (763.56) for N_2 plasma at 50 W for 10 minute exposure and in case of O_2 plasma, the higher vigour index (446.36) was observed at 30W for 10 minute exposure . The results indicated that among the cold plasma treatments, irradiation with N_2 plasma was more effective due to higher value of germination index and vigour index than the treatments with O_2 plasma. Moreover, it was observed that increase in discharge power and time of exposure did not markedly influence the germination parameters as the optimum germination characteristics was exhibited even at 30W power discharge and 10 minute exposure time Effectiveness of N_2 plasma might be due to the fact that nitrogen present in plasma play an important role in the intensification of the biological processes whereas atomic oxygen and OH radicals generated in plasma are the most probable sterilizing agents, as suggested by Filatova *et al.*, 2011.

Table 1: Impact of cold plasma irradiation on the germination characteristics of green gram under PEG induced water stress

Treatment	Power Discharge	Time of Exposure	Germination Potential (%)	Germination Rate (%)	Germination Index	Vigour Index
Control			93.33 ± 3.09^{d}	96.67 ± 0.45^{e}	$28.25 \pm 5.99^{a,b}$	298.32 ± 7.07^{d}
Water stressed			76.67 ± 5.09^{a}	83.33 ± 2.64^{a}	23.75 ± 3.89^{e}	147.96 ± 7.00^{a}
N_2 Plasma	30W	10 min	96.67 ± 1.91^{e}	100.00 ± 0.00^{e}	29.25 ± 5.09^{a}	$422.95 \pm 8.01^{g,h}$
		20 min	93.33 ± 1.92^{d}	$93.33 \pm 1.92^{c,d}$	$28.00 \pm 1.54^{b,c}$	$384.44 \pm 6.04^{g,h}$
		30 min	90.00 ± 4.99^{c}	90.00 ± 4.99^{c}	27.00 ± 3.81^{d}	206.01 ± 6.01^{b}
	50W	10 min	96.67 ± 2.96^{e}	100.00 ± 0.00^{e}	29.30 ± 5.94^{a}	763.56 ± 6.99^{m}
		20 min	96.67 ± 2.19^{e}	100.00 ± 0.00^{e}	29.50 ± 4.98^{a}	653.72 ± 5.14^{l}
		30 min	93.33 ± 2.83^{e}	93.33 ± 2.83^{e}	$28.00 \pm 2.64^{b,c}$	654.08 ± 3.20^{l}
	70W	10 min	83.33 ± 2.47^{b}	90.00 ± 4.19^{b}	25.75 ± 5.00^{d}	559.55 ± 8.75^{k}
		20 min	96.67 ± 6.06^{e}	100.00 ± 0.00^{e}	29.20 ± 1.89^{a}	496.11 ± 6.94^{j}
		30 min	96.67 ± 1.45^{e}	96.67 ± 1.45^{e}	29.00 ± 5.13^{a}	461.11 ± 8.96^{j}
O_2 Plasma	30W	10 min	96.67 ± 4.85^{e}	100.00 ± 0.00^{e}	29.25 ± 2.43^{a}	446.36 ± 5.52^{j}
		20 min	83.33 ± 1.51^{b}	86.67 ± 0.81^{a}	$25.25 \pm 1.06^{d,e}$	$406.53 \pm 4.74^{h,i}$
		30 min	96.67 ± 3.08^{e}	96.67 ± 3.08^{e}	$28.00 \pm 5.33^{a,b}$	$317.24 \pm 4.97^{e,f}$
	50W	10 min	83.33 ± 3.98^{b}	$90.00 \pm 2.65^{b,c}$	$25.75 \pm 5.00^{c,d}$	279.65 ± 9.00^{c}
		20 min	96.67 ± 4.89^{e}	100.00 ± 0.00^{e}	28.33 ± 4.82^{a}	313.85 ± 3.00^{e}
		30 min	96.67 ± 1.18^{e}	96.67 ± 1.18^{e}	$29.00 \pm 5.32^{a,d}$	$367.64 \pm 6.82^{e,f}$
	70W	10 min	76.67 ± 4.89^{a}	83.33 ± 7.45^{a}	24.25 ± 2.83^{e}	$376.60 \pm 7.00^{e,f,g}$
		20 min	90.00 ± 7.40^{c}	$90.00 \pm 7.40^{b,c}$	$27.00 \pm 3.55^{c,d}$	290.52 ± 7.80^{c}
		30 min	96.67 ± 3.31^{e}	$96.67 \pm 3.31^{e,d}$	$29.00 \pm 1.68^{a,b}$	232.87 ± 6.00^{c}

All the data are expressed as mean ± standard deviations. Means values with the different superscript letters in a column differ significantly ($P < 0.05$).

It was reported by Sadhu *et al.*, 2017 that Cold plasma significantly increased the germination rate by 36.2%, radical root length by 20% and conductivity of seeds by 102% when compared to the control samples in mung bean. Huang, *et al.*, 2010, opined that plasma treatments enhance the ability of plants to cope with biotic and abiotic stress, such as drought stress. Positive influence of the cold plasma treatment on the germination rate of bean seeds and on the kinetics of germination was recorded for the alfalfa and trifolium seeds under the conditions of drought stress. Under mild drought stress and harsh drought stress, the germination rate of non-treated seeds was significantly reduced by 6% and by 10%, respectively, compared with the well-watered seeds. The cold plasma treatment significantly increased the germination rate by 10% in beans compared to the mild drought-stressed and harsh drought-stressed non-treated seeds. (Ling *et al*., 2015). Ling *et al*, 2015 also investigated the effect of cold plasma oilseed rape under drought stress in a drought-sensitive (Zhongshuang 7) and drought-tolerant cultivar (Zhongshuang 11) and it was observed that cold plasma treatment significantly improved the germination rate by 6.25% in Zhongshuang 7, and 4.44% in Zhongshuang 11 under drought stress. The formation of reactive oxygen species and reactive nitrogen species in the plasma could be mainly responsible for increase in seed germination rate. It was reported that of all these reactive species formed in the PAW, the nitrate ions serve as the fertilizer and NO radical breakdown dormancy which enhance seed germination rate. Bormashenko *et al.*, 2012 suggested that plasma etching or scratching effect on the seed coat would have increased the hydrophilicity of seeds. Similar increase in mung bean seed germination and seedling growth was reported by Zhou *et al.*, 2016 on the effect of Atmospheric-Pressure N_2, He, Air, and O_2 microplasmas.

3.2. Seedling growth:

3.2.1 Shoot and Root length:

The present study demonstrated that cold plasma treatment promoted seedling growth in green gram under drought stress which is evident from Figure 5-6. Effect of cold plasma treatment was more intense on root growth than shoot growth. It was found that shoot length and root length was increased in all cold plasma treated seeds on germination when compared to water stress induced PEG treatment whereas N_2 cold plasma exposure at 50 W for 10 minute was found to have the maximum shoot length (12.8 cm) and root length (13.26 cm) greater than that of water unstressed control and the increase in shoot length was found to be 54.2 %. Similarly the shoot length was decreased by 58.1% in water stressed treatment than water unstressed control. Moreover, among the treatments, N_2 plasma irradiated treatments exhibited higher shoot and root length than O_2 plasma irradiated treatments. However, there was no significant increase among the treatments on increasing the power and time of exposure. Ling *et al*, 2014 observed similar findings in the effect of cold plasma irradiation using Helium plasma discharge in the Soy bean seeds. Plasma treatment delivers sufficient quantities of exogenous H_2O_2 to mung bean seeds that may effectively increase the oxygen scavenging ability of the plant and that might be cause for increase in seed germination rate and promote the growth of mung bean seedlings. (Zhou *et al.*, 2016)

3.2.2. Shoot and Root dry weight:

In the present study, the impact of cold plasma treatment on dry weight of root and shoot in green gram under water stress was given in Figure 7-8. The dry root weight (86 mg) of N_2 plasma irradiated seeds at 50 W for 20 minutes was maximum than that of the water stressed treatment (15 mg) and in O_2 plasma irradiated seeds, the maximum dry weight of root (60.3 mg) was observed in 50W for 10 minutes exposure. Effect of cold plasma was more intense on root growth than shoot growth. Even the lateral root formation was more pronounced in the cold plasma treatments than control. These results are in agreement with Sera´*et al.*, 2010 who found that plasma had a greater effect on the dry weights of roots than shoots. Similarly the shoot dry weight was greater for all the cold plasma treated samples than water stressed treatment, however it was less than that of water unstressed control. On comparison with water stressed treatment, the shoot dry weight of N_2 cold plasma treatment at 50 W for 30 minute exposure was increased by 85.8 % and among the O_2 plasma treatments, 70W for 10 minute exposure was found to have increased shoot weight of 76.1 %. However, no significant increase in root and shoot dry weight was observed between treatments based on increase in power discharge or time of exposure.

The result was similar to work of Ling *et al*, 2015 who reported that cold plasma treatment markedly improved seedling growth under well-watered and drought stress conditions. The dry weight of shoot and root, length of shoot and root and lateral root number of Zhongshuang 7 treated by cold plasma were significantly increased by 16.67% , 20.22% , 42.72% , 19.09% and 29.12% and those of Zhongshuang 11 were improved by 15.00% , 15.16%, 30.09%, 19.83% and 38.14% respectively, compared to the drought-stressed seedlings.

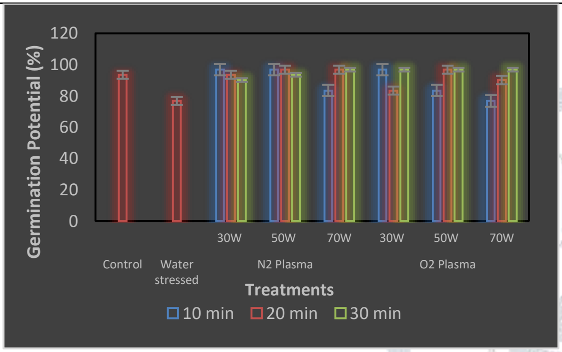	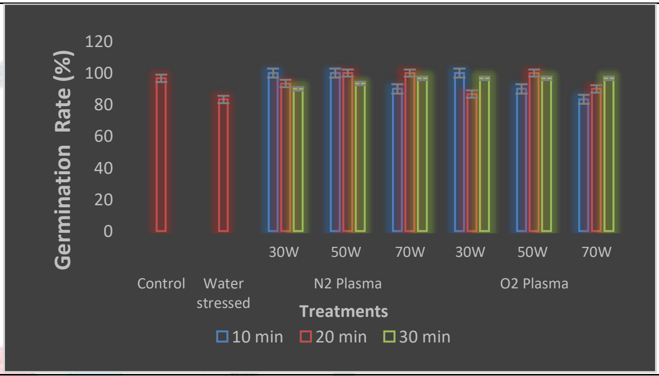
Fig. 1. Impact of cold plasma treatment on the germination potential (%) in green gram under PEG induced water stress as a function of feed gas, power and time of exposure	Fig. 2. Impact of cold plasma treatment on the germination rate (%) in green gram under PEG induced water stress as a function of feed gas, power and time of exposure
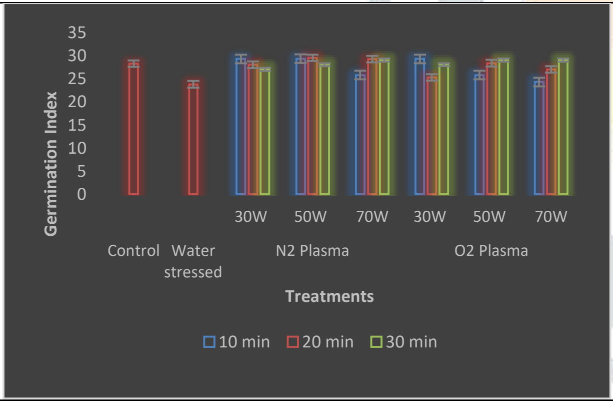	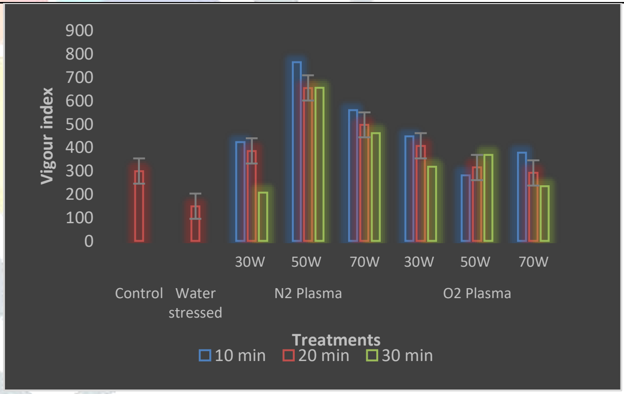
Fig. 3. Impact of cold plasma treatment on the germination index in green gram under PEG induced water stress as a function of feed gas, power and time of exposure	Fig. 4. Impact of cold plasma treatment on the vigour index in green gram under PEG induced water stress as a function of feed gas, power and time of exposure

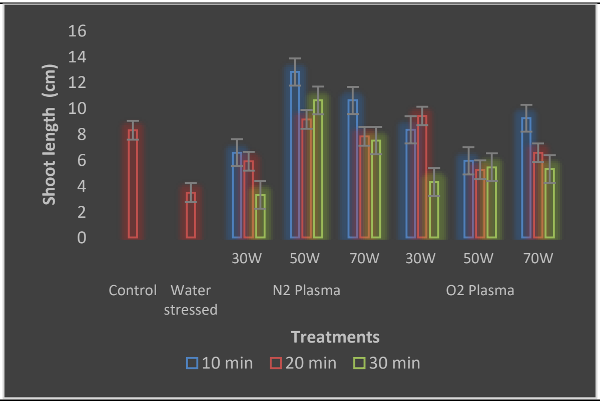	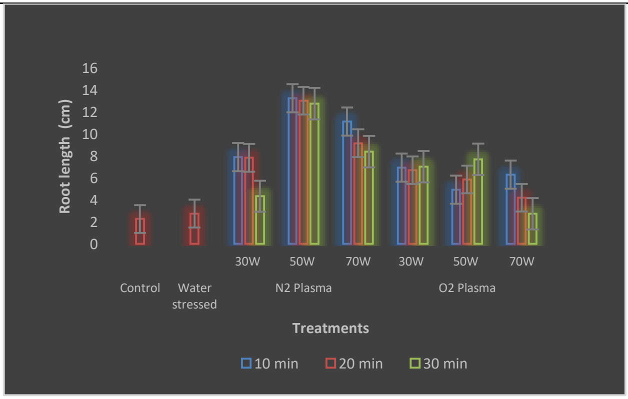

Fig. 5. Impact of cold plasma treatment on the shoot length in green gram under PEG induced water stress as a function of feed gas, power and time of exposure

Fig. 6. Impact of cold plasma treatment on the root length in green gram under PEG induced water stress as a function of feed gas, power and time of exposure

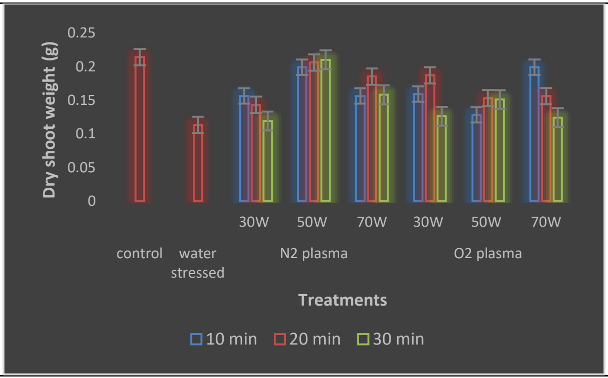

Fig. 7. Impact of cold plasma treatment on the dry weight of shoot in green gram under PEG induced water stress as a function of feed gas, power and time of exposure

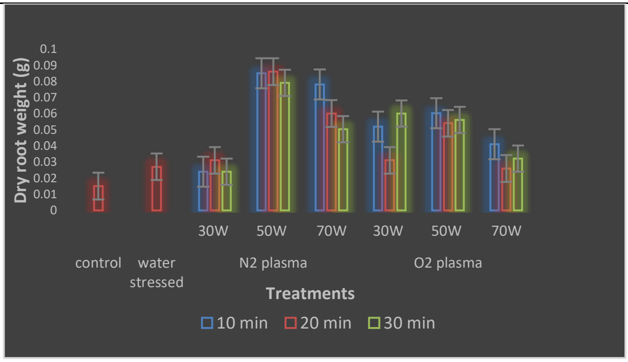

Fig. 8. Impact of cold plasma treatment on the dry weight of root in green gram under PEG induced water stress as a function of feed gas, power and time of exposure

IV. CONCLUSION

In this study, seed germination and seedling growth rates of green gram under water stress induced by Polyethylene glycol. was carried out by using N$_2$ and O$_2$ cold plasma irradiation. Compared to O$_2$ and N$_2$ plasma treatment, N$_2$ plasma treatment at 30W for 10 minutes was more effective in enhancing seed germination and N$_2$ plasma at 50 W 10 minute treatment exhibited maximum vigour index and seedling growth under drought stress. Even though the germination was enhanced at 30W, the treatments with further increase in power level 50W promoted maximum seedling growth and vigour. Reactive Oxygen Species and Reactive Nitrogen Species generated by plasma in solution might play a critical role in the germination and growing process. The increase in seed roughness or etching caused by bombardment of reactive species might be the reason for increase in hydrophilicity of seeds. The results of the study demonstrated the feasibility and advantages of innovative and novel eco- agro technique of cold plasma irradiation and its application to seed treatment and amelioration of biotic and abiotic stress.

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