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Effect of Seed Coating Polymer and Micronutrients on Stomatal Conductance and Resistance at Different Growth Stages of Pigeonpea

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study aims to evaluate the effect of seed coating polymer and micronutrients on stomatal conductance and resistance at different growth stages of pigeonpea.
Place of Study: Field experiment was carried out during *kharif* 2014 at Main Agricultural Research Station, College of Agriculture, University of Agricultural Sciences, Raichur, India.
Methodology: A randomised block design was applied to determine the effect of seed coating polymer and micronutrients on stomatal conductance and resistance at different growth stages of pigeonpea. The experiment consisted of 16 different treatments laid out in a randomised block design with three replications. The micronutrients were applied to the seed either individually or in combination as per the studied treatments.
Results and Discussion: The study result revealed improved physiological parameters due to seed polymerisation with micronutrients and foliar spray, there was a significant difference in the seed yield of treated treatments with that of the untreated control. Physiological observations on Stomatal

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conductance (M mol/m^2s) and resistance (m^2s/mol) of five randomly tagged plants were recorded by using leaf porometer (SC-1 porometer, Decagon Devices, Pullman, WA, USA) at 45, 90 and 120 DAS and finally seed yield was recorded, analysed statistically to study the effect of seed coating polymer and micronutrients on stomatal conductance and resistance at different growth stages of pigeonpea. Seed coating with polymer, micronutrients and foliar spray had significant ($p<0.05$) influence on stomatal conductance, resistance and seed yield of pigeonpea. Stomatal conductance differed significantly due to seed polymerisation with micronutrients and foliar spray at all the growth stages.

Conclusion: Micronutrients *viz*., zinc, boron and potassium molybdate in combination along with standardised seed coating polymer significantly influenced the physiological parameters *viz.,* stomatal conductance and resistance of leaf thus enhancing the photosynthetic efficiency, finally helping in the better establishment of seedlings and higher seed yield.

Keywords: Micronutrients; Pigeonpea, seed polymerisation, stomatal conductance and resistance.

1. INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp., Family-Fabaceae], is one of the major pulse crops of the tropics and sub-tropics, grown in approximately 50 countries in Asia, Africa and the Americas, mostly as an intercrop with cereals. It is commonly known as pigeonpea, red gram, tur, arhar, tuvarica, Congobean, thogari or gandul in India. Pigeonpea pods are consumed as green vegetable in many countries. Dry seeds of pigeonpea are consumed as split dhal. Pigeonpea is also used as a ration for milch cattle. Its straw is also palatable and green leaves may be used as fodder. Sticks of pigeonpea are used for various purposes such as thatch and basket making, etc. Recently its use as a fodder crop has increased. Seed and Fodder contains approx, 20-22% protein. Seeds are rich in Iron, iodine, and essential amino acids like Lycine, Cystine and Arginine [1]. Pigeonpea being a leguminous plant is capable of fixing atmospheric nitrogen and thereby restore a lot of nitrogen in the soil. It is well recognised as a valuable source of dietary proteins. In addition to its nutritional value, it also has a unique property of maintaining and restoring soil fertility through biological nitrogen fixation and improvement of physical properties of the soil by virtue of its deep root system. Pigeonpea has several advantages over other leguminous crops for broad-scale agricultural production. These include drought tolerance, logging and shattering resisting which allow the possibility of rationing. Pigeonpea is grown throughout southern Asia including India, Myanmar, China and Nepal. About 95% production of pigeonpea is from South Asia, 90% of which belongs to India. It is also grown in parts of Africa, USA and has recently been introduced in Australia [2]. In India, the major pigeon pea growing states are Maharashtra, Uttar Pradesh, Karnataka, Madhya Pradesh, Andhra Pradesh and Gujarat. Having the wide adaptability to varying agro climatic condition, it is cultivated all over the country with the exception of the areas which are neither excessively wet or experience severe frost [3]. Its deep root system imports resistance to drought. In India, it is the second most important pulse crop after chickpea. India is the largest producer and consumer of pulses in the world. It is cultivated in an area of about 4.49 m ha and production of about 2.92 mt with the productivity of 628 kg/ha. In Karnataka, pigeonpea is grown in an area of 8, 94, 547 ha with the production of 5, 30,065 tonnes with the productivity of 629 kg/ha [3]. It is largely grown in the northern parts of the state. The five major pigeonpea producing districts are Gulbarga, Bijapur, Bidar, Yadgir and Raichur. Pigeonpea contains about 23.6 per cent protein, which is almost three times that of cereals. Pigeonpeas are popular food in developing tropical countries. Nutritious and wholesome, the green seeds (and pods) serve as a vegetable. Ripe seeds are a source of flour, used split (dhal) in soups or eaten with rice.

In spite of its nutritional importance, the production of pigeonpea is very low even in the era of the green revolution. In recent years, there has been a significant decline in the pigeonpea production in India, leading to an increasing in price and reduction in per capita availability. The availability of pulses in India is 35 gm/day/capita [4] as against the minimum requirement of 80 gm/day. Among the many factors responsible for the poor productivity of the pigeonpea, inadequate supply of micronutrients in addition to macronutrients is one of them. The deficiency of these micronutrients has been very pronounced under multiple cropping systems due to excess removal by high yielding varieties and hence their exogenous supplies are urgently required.

Micronutrients are very efficient in minute quantities to produce optimum effects. Micronutrients play a vital role in enhancing crop productivity. Among micronutrients, the deficiency of Zn is widespread in Indian soils. Mo deficiency, by and large, is associated with acid soils. Intensification of agriculture with high yielding crop varieties, continuous use of high analysis chemical fertilisers, restricted supply of organic manures and negligible crop residue return to the soil led to micronutrient deficiency. While the micronutrients are required relatively in smaller quantities but they are as important as macronutrients. If any of these elements is lacking in the soil or not adequately balanced with other nutrients, growth suppression or even complete inhibition may occur [5]. Micronutrients often act as cofactors in enzyme systems and participate in redox reactions, in addition to having several other vital functions in plants. Most importantly, micronutrients are involved in the key physiological processes of photo-synthesis and respiration and their deficiency can impede these vital physiological processes thus limiting yield gain in many crops.

These micronutrients may be supplied to the plants through soil application, foliar spray or seed treatment. Micronutrient application through seed treatment improves the stand establishment, advances phenological events, increases yield and micronutrient contents in grain in most of the crops. In many cases, micronutrient application through seed treatment performed better or similar to other application methods [6, 7]. Being an easy and cost effective method, seed treatment by polymer coating offer an attractive option for resource-poor farmers through its pronounced effect during the early stage of seedling establishment [8].

Stomatal conductance estimates the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata as determined by the degree of stomatal aperture (and therefore the physical resistance to the movement of gases between the air and the interior of the leaf). Hence, it is a function of the density, size and degree of opening of the stomata; with more open stomata allowing greater conductance, and consequently indicating that photosynthesis and transpiration rates are potentially higher. The handheld porometer provides rapid measurement of leaf stomatal conductance in irrigated trials, though it is not a recommended measurement under water stress (unless very mild) as the stomata are generally closed [9]. Keeping in view the importance of above facts, the present investigation was carried out to study the effect of seed coating polymer and micronutrients on stomatal conductance and resistance at different growth stages of pigeonpea.

2. MATERIALS AND METHODS

A field experiment was conducted in the Main Agricultural Research Station, University of Agricultural Sciences, Raichur, India during *kharif* 2014 in a randomised block design to study the effect of seed coating polymer and micronutrients on stomatal conductance and resistance at different growth stages of pigeonpea. The experiment consisted of 16 different treatments laid out in randomized block design with three replications (Table 1).

Table 1. List of treatments showing respective experimental design

Sl No.	Treatments	Experimental setup
1	T_1	Potassium molybdate @ 2 g per kg of seed
2	T_2	Potassium molybdate @ 4 g per kg of seed
3	T_3	$ZnSO_4$ @ 2 g per kg of seed
4	T_4	$ZnSO_4$ @ 4 g per kg of seed
5	T_5	Boron @ 2 g per kg of seed
6	T_6	Boron @ 4 g per kg of seed
7	T_7	Potassium molybdate + $ZnSO_4$ (each @ 2 g / kg of seed)
8	T_8	Potassium molybdate + $ZnSO_4$ (each @ 4 g / kg of seed)
9	T_9	$ZnSO_4$ + Boron (each @ 2 g / kg of seed)
10	T_{10}	$ZnSO_4$ + Boron (each @ 4 g / kg of seed)
11	T_{11}	Potassium molybdate + Boron (each @ 2 g / kg of seed)
12	T_{12}	Potassium molybdate + Boron (each @ 4 g / kg of seed)
13	T_{13}	Potassium molybdate + $ZnSO_4$ + Boron (each @ 2 g / kg of seed)
14	T_{14}	Potassium molybdate + $ZnSO_4$ + Boron (each @ 4 g / kg of seed)
15	T_{15}	Only polymer
16	T_{16}	Absolute control

Fig. 1. Pigeonpea seeds coated with polymer and micronutrients

Fig. 2. Leaf Porometer (SC-1 porometer, Decagon Devices, Pullman, WA, USA) used during the experiment

The micronutrients were applied to the seed either individually or in combination as per the above treatments by using 6 ml polymer (Disco Agro DC Red L- 603 procured from Incotec Pvt. Ltd. Ahmedabad, Gujarat) dissolved in 45 ml water per kg of seed in a rotary seed coating machine (Fig. 1).

The coated seeds were properly dried in shade and sown in three replications with randomised block design with spacing of 90 x 30 cm. In addition to these treatments, two foliar sprays at an interval of 10 days during flowering stage (75 and 85 DAS) were applied either individually or in combination as per the treatments (0.5% + 0.1% + 0.2% ZnSO4 and potassium molybdate in EDTA form) respectively. The biometric observations on stomatal conductance and resistance parameters were recorded at three stages of the plant growth *viz*., vegetative (45 DAS), flowering (90 DAS) and pod filling (120 DAS) stages. For recording such observations, five plants at random from net plot area were selected and tagged in each plot. Leaf stomatal conductance and resistance was measured by using leaf porometer (SC-1 porometer, Decagon Devices, Pullman, WA, USA) (Fig. 2) and seed yield per hectare was recorded at harvest.

2.1 Statistical Analysis

The statistical analysis was done as per the procedure described by Panse & Sukhatme [10].

3. RESULTS AND DISCUSSION

The stomatatal conductance is the measure of the rate of passage of carbon dioxide (CO_2) entering, or water vapour exiting through the stomata of a leaf. Stomata are small pores on the top and or bottom of a leaf that are responsible for taking in and expelling CO_2 and moisture from and to the outside air. The rate of stomatal conductance, or its inverse, stomatal resistance, is directly related to the boundary layer resistance of the leaf and the absolute concentration gradient of water vapour from the leaf to the atmosphere. It is under direct biological control of the leaf through the use of guard cells, which surround the stomatal pore [11]. The turgor pressure and osmotic potential of guard cells is directly related to the stomatal conductance [12]. Stomatal conductance is a function of stomatal density, stomatal aperture, and stomatal size [13]. Stomatal conductance is integral to leaf level calculations of transpiration. Stomatal conductance is a measure of the degree of stomatal opening and can be used as an indicator of plant water status. Stomatal conductance is related to leaf water potential by feedback processes. Reductions in stomatal conductance prevent further decreases in water potential by reducing transpiration; also, reductions in water potential can induce stomatal closure, resulting in lowered stomatal conductance. Stomatal conductance can be measured with both dynamic and steady-state diffusion porometers. Seed coating with polymer, micronutrients and foliar spray had significant ($p<0.05$) influence on stomatal conductance, resistance and seed yield of pigeonpea.

Stomatal conductance differed significantly due to seed polymerisation with micronutrients and foliar spray at all the growth stages (Table 2). The treatment T_{13} recorded significantly highest stomatal conductance (568.6 M mol/m^2s, 891.6 M mol/m^2s and 860.7 M mol/m^2s) at 45, 90 and 120 DAS, respectively. It was followed by T_{14} (542.7 M mol/m^2s) at 45 DAS. Similar trend was observed at 90 DAS (878.2 M mol/m^2s) and 120 DAS (847.2 M mol/m^2s). However, control (T_{16}) recorded the lowest stomatal conductance (343.3 M mol/m2s, 649.6 M mol/m^2s and 613.3 M mol/m^2s) at 45 DAS, 90 DAS and 120 DAS, respectively. Significantly lowest stomatal resistance (2.28 m^2s/mol, 2.17 m^2s/mol and 2.21 m^2s/mol) was recorded in the treatment T_{13} at 45, 90 and 120 DAS, respectively (Table 3). However, T_{13} was followed by T_{14} (2.50 m^2s/mol, 2.37 m^2s/mol and 2.49 m^2s/mol) at 45, 90 and 120 DAS, respectively. Whereas, control (T_{16}) recorded highest stomatal resistance (3.81 m^2s/mol, 3.11 m^2s/mol and 3.62 m^2s/mol) at 45 DAS, 90 DAS and 120 DAS, respectively.

The significant increase in stomatal conductance might be attributed to the physiological functions of zinc and K^+ ion in the opening and closing of the stomata which in turn affected the CO_2 uptake and transpiration losses. Hu and Sparks [14] reported a significant decrease in stomatal conductance in Zn-deficient ‘Stuart’ pecan (*Carya illinoensis*) leaves. However, molybdenum in combination with zinc also contributed to the higher stomatal conductance. It might be due to the role of molybdenum in N_2 fixation, it indicates that nitrogen helps in reducing the resistance of stomata in leaf. Since, the nitrogen supplied plants have wide stomatal aperture than the nitrogen deficient plants. These results were similar to that of Fois et al. [15] who concluded that leaf resistance decreased from lower (40 kg N/ha) to higher level (120 kg N/ha) of N fertilisation in wheat and [16] in Kenaf (*Hibiscus cannabinus* L.) plant. Younes et al. [17] studied biofertilisers and zinc effects on some physiological parameters of triticale under water limitation condition and the results of measurement of stomatal conductance showed that the stomatal conductance decreased under water limitation. Further under severe water limitation, application of bio fertilisers as F_3 and nano zinc oxide as Zn_3 increase stomatal conductance about 34.6% in flowering stage, 42.1% in heading stage and 35.4% in grain filling stage in comparison with F_0 and Zn_0 in the same water-limitation level. Parma et al. [18] also reported an involvement of Zn in stomatal opening, possibly as a constituent of the enzyme CA and/or as a factor in maintaining membrane integrity and K^+ uptake.

As a result of improved physiological parameters due to seed polymerisation with micronutrients and foliar spray there was a significant difference in the seed yield of treated treatments with that of the untreated control. The treatment T_{13}

[Potassium molybdate + $ZnSO_4$ + boron (each @ 2g / kg seed)] along with two foliar sprays of potassium molybdate (0.1%) + zinc sulphate (0.5%) in EDTA form + borax (0.2%) produced higher seed yield per hectare (16.30 q) which was found to be superior over all the treatments. Whereas, the lowest seed yield per hectare (13.86 q/ha) was recorded in the control (T_{16}).

Table 2. Influence of seed polymerisation with micronutrients and foliar spray on stomatal conductance (M mol/m^2s) at different growth stages of pigeonpea

Treatments	Stomatal conductance (M mol/m^2s)			Seed yield (q)/ha
	45 Days	90 Days	120 Days	
T_1: Potassium molybdate @ 2 g per kg of seed	419.3	754.5	721.8	14.20
T_2: Potassium molybdate @ 4 g per kg of seed	445.7	784.2	754.6	14.46
T_3: $ZnSO_4$ @ 2 g per kg of seed	388.5	678.2	648.6	14.10
T_4: $ZnSO_4$ @ 4 g per kg of seed	422.9	763.9	733.3	14.15
T_5: Boron @ 2 g per kg of seed	409.7	712.2	681.6	14.10
T_6: Boron @ 4 g per kg of seed	443.6	783.4	751.3	14.30
T_7: Potassium molybdate + $ZnSO_4$ (each @ 2 g / kg of seed)	478.3	846.6	789.2	14.95
T_8: Potassium molybdate + $ZnSO_4$ (each @ 4 g / kg of seed)	476.8	814.3	786.0	14.92
T_9: $ZnSO_4$ + Boron (each @ 2 g / kg of seed)	474.1	812.9	782.4	14.86
T_{10}: $ZnSO_4$ + Boron (each @ 4 g / kg of seed)	472.1	810.7	780.1	14.20
T_{11}: Potassium molybdate + Boron (each @ 2 g / kg of seed)	536.6	872.9	841.7	15.17
T_{12}: Potassium molybdate + Boron (each @ 4 g / kg of seed)	531.4	869.0	839.2	15.06
T_{13}: Potassium molybdate + $ZnSO_4$ + Boron (each @ 2 g / kg of seed)	568.6	891.6	860.7	16.30
T_{14}: Potassium molybdate + $ZnSO_4$ + Boron (each @ 4 g / kg of seed)	542.7	878.2	847.2	15.20
T_{15}: Only polymer	372.5	665.5	635.1	14.03
T_{16}: Absolute Control	343.3	649.6	613.3	13.86
Mean	**457.9**	**784.4**	**754.2**	**14.62**
S.Em.±	1.16	1.19	1.10	0.14
CD (P = 0.05)	3.39	3.45	3.19	0.40

Table 3. Influence of seed polymerisation with micronutrients and foliar spray on stomatal resistance (m^2s/mol) at different growth stages pigeonpea

Treatments	Stomatal resistance (m^2s/mol))			Seed yield (q)/ha
	45 Days	90 Days	120 Days	
T_1: Potassium molybdate @ 2 g per kg of seed	3.45	3.03	3.11	14.20
T_2: Potassium molybdate @ 4 g per kg of seed	3.02	2.86	2.98	14.46
T_3: $ZnSO_4$ @ 2 g per kg of seed	3.68	3.00	3.11	14.10
T_4: $ZnSO_4$ @ 4 g per kg of seed	3.42	2.96	3.04	14.15
T_5: Boron @ 2 g per kg of seed	3.65	3.00	3.09	14.10
T_6: Boron @ 4 g per kg of seed	3.38	2.93	2.99	14.30
T_7: Potassium molybdate + $ZnSO_4$ (each @ 2 g / kg of seed)	2.64	2.51	2.57	14.95
T_8: Potassium molybdate + $ZnSO_4$ (each @ 4 g / kg of seed)	2.71	2.60	2.66	14.92
T_9: $ZnSO_4$ + Boron (each @ 2 g / kg of seed)	2.75	2.68	2.71	14.86
T_{10}: $ZnSO_4$ + Boron (each @ 4 g / kg of seed)	2.98	2.75	2.86	14.20
T_{11}: Potassium molybdate + Boron (each @ 2 g / kg of seed)	2.54	2.48	2.51	15.17
T_{12}: Potassium molybdate + Boron (each @ 4 g / kg of seed)	2.54	2.39	2.46	15.06
T_{13}: Potassium molybdate + $ZnSO_4$ + Boron (each @ 2 g / kg of seed)	2.28	2.17	2.21	16.30
T_{14}: Potassium molybdate + $ZnSO_4$ + Boron (each @ 4 g / kg of seed)	2.50	2.37	2.49	15.20
T_{15}: Only polymer	3.76	3.11	3.56	14.03
T_{16}: Absolute Control	3.81	3.11	3.62	13.86
Mean	**3.07**	**2.75**	**2.87**	**14.62**
S.Em.±	0.03	0.03	0.03	0.14
CD (P = 0.05)	0.09	0.08	0.08	0.40

It can be correlated that with enhanced stomatal conductance wherein, the uptake of carbon dioxide might be more leading to higher production of carbohydrates and thereby translocation of these metabolites to root nodules. Due to enhancement of root nodules nitrogen assimilation might be higher thereby led to increased number of branches which might be due to translocation of metabolites and carbohydrates from photo-synthetically active leaf from branches to the developing pod and in turn accumulation of these carbohydrates in seed resulted in increased test weight, which finally increased the seed yield/ hectare.

4. CONCLUSION

Micronutrients *viz.,* zinc, boron and potassium molybdate in combination along with standardised seed coating polymer significantly influenced the physiological parameters viz., stomatal conductance and resistance of leaf thus enhancing the photosynthetic efficiency, finally helping in the better establishment of seedlings and higher seed yield. The significant increase in stomatal conductance might be attributed to the physiological functions of zinc and K^+ ion in the opening and closing of the stomata which in turn affected the CO_2 uptake and transpiration losses. Seed polymerisation of pigeonpea seeds with the combination of micronutrients (potassium molybdate + $ZnSO_4$ + boron each at 2 g per kg of seed) with two foliar sprays at an interval of 10 days during flowering stage found to be optimum dose for pigeonpea cultivation during kharif season.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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