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

ABSTRACT

Aim: to study 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.

Methodology: The experiment consisted of 16 different treatments wherein fresh seeds of pigeonpea were treated with different micronutrients *viz.*, Potassium molybdate (2 or 4 ml per kg of seed), ZnSO₄ (2 or 4 ml per kg of seed) and boron (2 or 4 ml per kg of seed) either individually or in combinations by using polymer at the rate of 6 ml per kg of seed which was standardized in the laboratory experiment. In addition, two foliar sprays as per the treatments either individually or in combination at an interval of 10 days during flowering stage (75 and 85 DAS) were given [Potassium molybdate (0.1 %), (Zinc sulphate (0.5 %) in EDTA form, Borax (0.2 %)]. Physiological observations on Stomatal conductance (M mol/m²s) and resistance (m²s/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.

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

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Keywords: [Micronutrients; Pigeonpea, seed polymerization, stomatal conductance and resistance] **1. INTRODUCTION**

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14 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, 15 16 mostly as an intercrop with cereals. It is commonly known as pigeonpea, red gram, tur, arhar, 17 tuvarica, Congobean, thogari or gandul in India. Pigeonpea pods are consumed as green vegetable in 18 many countries. Dry seeds of pigeonpea are consumed as split dhal. Pigeonpea is also used as ration 19 for milch cattle. Its straw is also palatable and green leaves may be used as fodder. Sticks of 20 pigeonpea are used for various purposes such as thatch and basket making, etc. Recently its use as 21 a fodder crop has increased. Seed and Fodder contains approx, 20-22% protein. Seeds are rich in 22 Iron, iodine, and essential amino acids like Lycine, Cystine and Arginine. Pigeonpea being a 23 leguminous plant is capable of fixing atmospheric nitrogen and thereby restore lot of nitrogen in the 24 soil. It is well recognized as a valuable source of dietary proteins. In addition to its nutritional value it 25 also has a unique property of maintaining and restoring soil fertility through biological nitrogen fixation 26 and improvement of physical properties of the soil by virtue of its deep root system. Pigeonpea has 27 several advantages over other leguminous crops for broad scale agricultural production. These 28 include drought tolerance, logging and shattering resisting which allow the possibility of rationing. 29 Pigeonpea is grown throughout the southern Asia including India, Myanmar, China and Nepal. About 30 95% production of pigeonpea is from south Asia, 90% of which belongs to India. It is also grown in 31 parts of Africa, USA and has recently been introduced in Australia. In India the major pigeonpea 32 growing states are Maharashtra, Uttar Pradesh, Karnataka, Madhya Pradesh, Andhra Pradesh and 33 Gujarat. Having the wide adaptability to varying agro climatic condition, it is cultivated all over the 34 country with the exception of the areas which are neither excessively wet or experience severe frost. 35 Its deep root system imports resistance to drought. In India, it is the second most important pulse crop 36 after chickpea. India is the largest producer and consumer of pulses in the world. It is cultivated in an 37 area of about 4.49 m ha and production of about 2.92 mt with productivity of 628 kg/ha. In Karnataka, 38 pigeonpea is grown in an area of 8, 94, 547 ha with the production of 5, 30,065 tonnes with the 39 productivity of 629 kg/ha [1]. It is largely grown in the northern parts of the state. The five major 40 pigeonpea producing districts are Gulbarga, Bijapur, Bidar, Yadgir and Raichur. Pigeonpea contains 41 about 23.6 per cent protein, which is almost three times that of cereals. Pigeon peas are popular food 42 in developing tropical countries. Nutritious and wholesome, the green seeds (and pods) serve as 43 vegetable. Ripe seeds are a source of flour, used split (dhal) in soups or eaten with rice.

44 In spite of its nutritional importance, the production of pigeonpea is very low even in the era of 45 green revolution. In recent years, there has been significant decline in the pigeonpea production in 46 India, leading to increase in price and reduction in per capita availability. The availability of pulses in 47 India is 35 gm/day/capita [2] as against the minimum requirement of 80 gm/day. Among the many 48 factors responsible for the poor productivity of the pigeonpea, inadequate supply of micronutrients in 49 addition to macronutrients is one of them. The deficiency of these micronutrients has been very 50 pronounced under multiple cropping systems due to excess removal by high yielding varieties and 51 hence their exogenous supplies are urgently required.

Micronutrients are very efficient in minute quantities to produce optimum effects. Micronutrients play 52 53 vital role in enhancing crop productivity. Among micronutrients, the deficiency of Zn is widespread in 54 Indian soils. Mo deficiency by and large is associated with acid soils. Intensification of agriculture with 55 high yielding crop varities, continuous use of high analysis chemical fertilizers, restricted supply of 56 organic manures and negligible crop residue return to the soil led to micronutrient deficiency. While, 57 the micronutrients are required relatively in smaller quantities but they are as important as 58 macronutrients. If any of these element is lacking in the soil or not adequately balanced with other 59 nutrients, growth suppression or even complete inhibition may occur [3]. Micronutrients often act as 60 cofactors in enzyme systems and participate in redox reactions, in addition to having several other 61 vital functions in plants. Most importantly, micronutrients are involved in the key physiological 62 processes of photosynthesis and respiration and their deficiency can impede these vital physiological 63 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 [4]. 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 [5].

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 resistances 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.

79 Keeping in view the importance of above facts, the present investigation was carried out to 80 study the effect of seed coating polymer and micronutrients on stomatal conductance and resistance 81 at different growth stages of pigeonpea.

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2. MATERIAL AND METHODS

A field experiment was conducted in the Main Agricultural Research Station, University of Agricultural Sciences, Raichur 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.

89 The experiment consisted of 16 different treatments viz., T₁: Potassium molybdate @ 2g per 90 kg of seed, T₂: Potassium molybdate @ 4g per kg of seed, T₃: ZnSO₄ @ 2g per kg of seed, T₄: ZnSO₄ 91 @ 4g per kg of seed, T_5 : Boron @ 2g per kg of seed, T_6 : Boron @ 4g per kg of seed, T_7 : Potassium 92 molybdate + ZnSO₄ (each @ 2g / kg of seed), T₈: Potassium molybdate + ZnSO₄ (each @ 4g / kg of 93 seed), T₉: ZnSO₄ + Boron (each @ 2g / kg of seed), T₁₀: ZnSO₄ + Boron (each @ 4g / kg of seed), 94 T₁₁: Potassium molybdate + Boron (each @ 2g / kg of seed), T₁₂: Potassium molybdate + Boron (each 95 @ 4g / kg of seed), T_{13} : Potassium molybdate + ZnSO₄ + Boron (each @ 2g / kg of seed), T_{14} : 96 Potassium molybdate + ZnSO₄ + Boron (each @ 4g / kg of seed), T₁₅: Only polymer and T₁₆: Absolute 97 control and laid out in randomized block design with three replicationsThe micronutrients were applied 98 to the seed either individually or in combination as per the above treatments by using 6 ml polymer 99 (Disco Agro DC Red L- 603 procured from Incotec Pvt. Ltd. Ahmedabad, Gujarat) dissolved in 45 ml 100 water per kg of seed in a rotary seed coating machine (Fig.1).





Fig.1. Pigeonpea seeds coated with polymer and micronutrients

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 106 10 days during flowering stage (75 and 85 DAS) were given either individually or in combination as 107 per the treatments (0.5 % + 0.1 % + 0.2%, respectively, ZnSO4 and potassium molybdate in EDTA 108 form). The biometric observations on stomatal conductance and resistance parameters were recorded 109 at three stages of the plant growth viz., vegetative (45 DAS), flowering (90 DAS) and pod filling (120 110 DAS) stages. For recording such observations, five plants at random from net plot area were selected 111 and tagged in each plot. Leaf stomatal conductance and resistance was measured by using leaf 112 porometer (SC-1 porometer, Decagon Devices, Pullman, WA, USA) (Fig. 2.). and seed yield per 113 hectare was recorded at harvest.



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

The statistical analysis was done as per the procedure described by [6].

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3. RESULTS AND DISCUSSION

120 121 Stomatatal conductance is the measure of the rate of passage of carbon dioxide (CO_2) 122 entering, or water vapour exiting through the stomata of a leaf. Stomata are small pores on the top 123 and or bottom of a leaf that are responsible for taking in and expelling CO₂ and moisture from and to 124 the outside air. The rate of stomatal conductance, or its inverse, stomatal resistance, is directly related 125 to the boundary layer resistance of the leaf and the absolute concentration gradient of water vapour 126 from the leaf to the atmosphere. It is under direct biological control of the leaf through the use of 127 guard cells, which surround the stomatal pore [7]. The turgor pressure and osmotic potential of guard 128 cells is directly related to the stomatal conductance [8]. Stomatal conductance is a function of stomatal 129 density, stomatal aperture, and stomatal size [9]. Stomatal conductance is integral to leaf level 130 calculations of transpiration (E). Stomatal conductance is a measure of the degree of stomatal 131 opening and can be used as an indicator of plant water status. Stomatal conductance is related to leaf 132 water potential (Ψ) by feedback processes. Reductions in stomatal conductance prevent further 133 decreases in water potential by reducing transpiration; also, reductions in water potential can induce 134 stomatal closure, resulting in lowered stomatal conductance. Stomatal conductance can be measured 135 with both dynamic and steady-state diffusion porometers.

Seed coating with polymer, micronutrients and foliar spray had significant influence on stomatal conductance, resistance and seed yield of pigeonpea. 138 Stomatal conductance differed significantly due to seed polymerization with micronutrients 139 and foliar spray at all the growth stages (Table 1.). The treatment T₁₃ recorded significantly highest stomatal conductance (568.6 M mol/m²s, 891.6 M mol/m²s and 860.7 M mol/m²s) at 45, 90 and 120 140 DAS, respectively. It was followed by T₁₄ (542.7 M mol/m²s) at 45 DAS. Similar trend was observed at 141 90 DAS (878.2 M mol/m²s) and 120 DAS (847.2 M mol/m²s). However, control (T₁₆) recorded the 142 143 lowest stomatal conductance (343.3 M mol/m2s, 649.6 M mol/m2s and 613.3 M mol/m2s) at 45 DAS, 144 90 DAS and 120 DAS, respectively. Significantly lowest stomatal resistance (2.28 m²s/mol, 2.17 145 m²s/mol and 2.21 m²s/mol) (Table 2.) was recorded in the treatment T₁₃ at 45, 90 and 120 DAS, 146 respectively. However, T₁₃ was followed by T₁₄ (2.50 m²s/mol, 2.37 m²s/mol and 2.49 m²s/mol) at 45, 90 and 120 DAS, respectively. Whereas, control (T₁₆) recorded highest stomatal resistance (3.81 147 148 m²s/mol, 3.11 m²s/mol and 3.62 m²s/mol) at 45 DAS, 90 DAS and 120 DAS, respectively.

149 The significant increase in stomatal conductance might be attributed to the physiological 150 functions of zinc and K^{+} ion in the opening and closing of the stomata which in turn affected the CO₂ 151 uptake and transpiration losses. [10] reported a significant decrease in stomatal conductance in Zn-152 deficient 'Stuart' pecan (Carya illinoensis) leaves. However, molybdenum in combination with zinc 153 also contributed to the higher stomatal conductance. It might be due to the role of molybdenum in N_2 154 fixation, it indicates that nitrogen helps in reducing the resistance of stomata in leaf. Since, the 155 nitrogen supplied plants have wide stomatal aperture than the nitrogen deficient plants. These results 156 were similar to that of [11] who concluded that leaf resistance decreased from lower (40 kg N/ha) to 157 higher level (120 kg N/ha) of N fertilisation in wheat and [12] in Kenaf (Hibiscus cannabinus L.) plant. 158 [13] studied bio fertilizers and zinc effects on some physiological parameters of triticale under water 159 limitation condition and the results of measurement of stomatal conductance showed that the stomatal 160 conductance decreased under water limitation. Further under severe water limitation, application of 161 bio fertilizers as F₃ and nano zinc oxide as Zn₃ increase stomatal conductance about 34.6% in 162 flowering stage, 42.1% in heading stage and 35.4% in grain filling stage in comparison with F_0 and 163 Zn₀ in the same water-limitation level. [14] also reported an involvement of Zn in stomatal opening, 164 possibly as a constituent of the enzyme CA and/or as a factor in maintaining membrane integrity and 165 K⁺ uptake.

As a result of improved physiological parameters due to seed polymerization with micronutrients and foliar spray there was significant difference in the seed yield of treated treatments with that of untreated control. The treatment T_{13} [Potassium molybdate + ZnSO₄ + 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 (T₁₆).

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Table 1. Influence of seed polymerization with micronutrients and foliar spray on stomatal conductance (M mol/m²s) at different growth stages of pigeonpea

Treatmente	Stoma (I	Seed yield		
Treatments	45 DAS	90 DAS	120 DAS	(q)/ha
T ₁ : Potassium molybdate @ 2g per kg of seed	419.3	754.5	721.8	14.20
T2: Potassium molybdate @ 4g per kg of seed	445.7	784.2	754.6	14.46
T ₃ : ZnSO ₄ @ 2g per kg of seed	388.5	678.2	648.6	14.10
T ₄ : ZnSO ₄ @ 4g per kg of seed	422.9	763.9	733.3	14.15

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409.7	712.2	681.6	14.10
443.6	783.4	751.3	14.30
478.3	846.6	789.2	14.95
476.8	814.3	786.0	14.92
474.1	812.9	782.4	14.86
472.1	810.7	780.1	14.20
536.6	872.9	841.7	15.17
531.4	869.0	839.2	15.06
568.6	891.6	860.7	16.30
542.7	878.2	847.2	15.20
372.5	665.5	635.1	14.03
343.3	649.6	613.3	13.86
457.9	784.4	754.2	14.62
1.16	1.19	1.10	0.14
3.39	3.45	3.19	0.40
	443.6 478.3 476.8 474.1 472.1 536.6 531.4 568.6 542.7 372.5 343.3 457.9 1.16	443.6 783.4 478.3 846.6 478.3 846.6 476.8 814.3 474.1 812.9 472.1 810.7 536.6 872.9 531.4 869.0 568.6 891.6 542.7 878.2 372.5 665.5 343.3 649.6 457.9 784.4 1.16 1.19	443.6 783.4 751.3 478.3 846.6 789.2 476.8 814.3 786.0 474.1 812.9 782.4 472.1 810.7 780.1 536.6 872.9 841.7 531.4 869.0 839.2 568.6 891.6 860.7 542.7 878.2 847.2 372.5 665.5 635.1 343.3 649.6 613.3 457.9 784.4 754.2 1.16 1.19 1.10

177 It can be correlated that with enhanced stomatal conductance wherein, the uptake of carbon 178 dioxide might be more leading to higher production of carbohydrates and thereby translocation of 179 these metabolites to root nodules. Due to enhancement of root nodules nitrogen assimilation might be 180 higher thereby led to increased number of branches which might be due to translocation of 181 metabolites and carbohydrates from photosynthetically active leaf from branches to the developing 182 pod and in turn accumulation of these carbohydrates in seed resulted in increased test weight, which 183 finally increased the seed yield/hectare.

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Table 2. Influence of seed polymerization with micronutrients and foliar spray on stomatal resistance (m²s/mol) at different growth stages pigeonpea

Treatments	stom	Seed yield		
Treatments	45 DAS	90 DAS	120 DAS	(q)/ha
T ₁ : Potassium molybdate @ 2g per kg of seed	3.45	3.03	3.11	14.20
T ₂ : Potassium molybdate @ 4g per kg of seed	3.02	2.86	2.98	14.46
T ₃ : ZnSO ₄ @ 2g per kg of seed	3.68	3.00	3.11	14.10
T ₄ : ZnSO ₄ @ 4g per kg of seed	3.42	2.96	3.04	14.15
T ₅ : Boron @ 2g per kg of seed	3.65	3.00	3.09	14.10
T ₆ : Boron @ 4g per kg of seed	3.38	2.93	2.99	14.30
T ₇ : Potassium molybdate + ZnSO ₄ (each @ 2g / kg of seed)	2.64	2.51	2.57	14.95
T ₈ : Potassium molybdate + ZnSO ₄ (each @ 4g / kg of seed)	2.71	2.60	2.66	14.92
T ₉ : ZnSO ₄ + Boron (each @ 2g / kg of seed)	2.75	2.68	2.71	14.86
T ₁₀ : ZnSO ₄ + Boron (each @ 4g / kg of seed)	2.98	2.75	2.86	14.20

T ₁₁ : Potassium molybdate + Boron (each @ 2g / kg of seed)	2.54	2.48	2.51	15.17
T ₁₂ : Potassium molybdate + Boron (each @ 4g / kg of seed)	2.54	2.39	2.46	15.06
T ₁₃ : Potassium molybdate + ZnSO ₄ + Boron (each @ 2g / kg of seed)	2.28	2.17	2.21	16.30
T ₁₄ : Potassium molybdate + ZnSO ₄ + Boron (each @ 4g / 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

4. CONCLUSION

Micronutrients viz., zinc, boron and potassium molybdate in combination along with standardized seed coating polymer significantly influenced the physiological parameters viz., stomatal conductance and resistance of leaf thus enhancing the photosynthetic efficiency, finally helping in better establishment of seedlings and higher seed yield. Seed polymerization (6 ml per kg of seed) of pigeonpea seeds with the combination of micronutrients namely, potassium molybdate + $ZnSO_4$ + boron each at 2g per kg of seed with two foliar sprays (0.1 % + 0.5 % + 0.2 % respectively, potassium molybdate and ZnSO₄ in EDTA form) at an interval of 10 days during flowering stage (75 and 85 DAS) found to be optimum dose for pigeonpea cultivation during kharif season.

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