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Effect of Biofertilizers [Azatobacter and Phosphate Solubilizing Bacteria] and their Combinations on Plant Height at maturity of *Brassica campestris*

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ABSTRACT: The investigation was carried out on *Brassica campestris*, two varieties cultivated with the treatment of two biofertillizers azatobacter chrococcum and phosphate solubilizing bacteria (PSB). In varieties ys-66 and k-88, effect of azatobacter15g inoculation showed 155.2±1.6 and 155.2±1.7 plant height respectively. PSB 15g inoculation showed 153.3±1.7 and 154.2±1.8 plant height respectively azatobacter +PSB 15g+15g inoculation showed 161.3± 2.0 and 162.2±1.8 respectively. In varities ys-66 and k-88, effect of azatobacter 15 g inoculation showed 10.3±1.7 and 11.1±1.4 number of branches per plant respectively. 15g PSB inoculation showed 9.7±1.3 and 9.6 ±1.1 respectively. Combined treatment of azatobacter + PSB showed 12.4±1.4 and 12.3±1.8 respectively. Control seeds of both varieties showed 146.3±4.2 and 146.3±3.2 plant height, 6.8±1.3 and 6.1±1.3 respectively.

Keywords: *Brassica campestris*, Azatobacter, Phosphate solubilizing bacteria (PSB), variety K-88, variety YS-66.

INTRODUCTION: The genus *Brassica* consists of over 150 species of annual or biennial herbs, several of which are cultivated as oil-seed crops (rape and mustard) or as vegetable crops (cabbage, cauliflower). A seeds of some yield the condiment, mustard. A few *Brassicas* furnish fodder and green manure.

This plant is natives of the North Temperate Zone, but pass into the sub-tropics and tropics as cold weather crop. A plant belonging to the species B. juncea found their way into India from China through North-Eastern route and their migration was independent of any Aryan incursion. The oil-yielding *Brassicas* which are predominantly cross- pollinated and *B.* campestris is self pollinated constitute a group, about which considerable confusion exists regarding their identification and nomenclature. Those extensively cultivated for the production of rape oil are: *B. napus*, *B. campestris* and *B. juncea* in China and Japan; *B. napus* and *B. praecox*. In Europe, America and India; and *B. juncea* and probably *B. napus* in Russia and in the Mediterranean areas.

Rape is a general term applied to seeds of Oleiferous *brassicas*, cultivated in many parts of the world as oilseed crops. Although several of these are distinct species or varieties, all yield oils with similar constants and composition, known as rape oil-sometimes also called colza oil. But the later term is now generally applied only to refined rape oil. A variety of *B. campestris* cultivated in the black sea region yields ravision oil, which is generally used for technical purposes. The chromosome number of the monogenomic species revealed that only six chromosome were distinctly different, the remaining belong homologous with one or another of the basic six (*Robbelen*, 1960 and *Aditya Kumar*, 2015). This evidence suggested an early evolution from a common progenitor species with chromosome number six. This crop originated from an extinct common ancestor with a basic chromosome number of n=5 or 6. The meitoic pairing in synthetic amphihaploid hybrids has demonstrated partial homology between A, B, C, E, S and R genome (Mizu Shima 1968).

Most of the species of *Brassica* are believed to originated in the mediterranean region. The primary centre of origin of B. *juncea* is believed to be central Asia-Himalayas with migration to secondary centres in India, China and Caucasus. The strong evidence of diversity in several aspects as reported by *Vaughan et al.*, (1963) indicate the independent hybridization of *B.* nigra with local species (n=10) or sub species in those areas, *B. carinata* arose from *B. nigra* by crossing with a *B. oleracea* forms in north-eastern Africa.

MATERIAL AND METHODS: The seeds of two common cultivars YS-66 and K-88 *Brassica campestris* L. Sarson were collected from Sehore Agriculture College, Sehore.

Bio-fertilizers (Azatobacter and PSB) were brought from M.P. Agro Industries, Bhopal and used in seed inoculation.The pure, healthy and good quality seeds of two cultivars of *Brassica campestris* sarson YS-66 and K-88 were obtained from pot and field experimental site. Seed samples were collected and finally cleaned and store the seeds of each pot and field as per treatments separately. (*Surender Pal*, 2014)The samples were preserved and used for analysis.

I. **Pot experiment:**

Pot size	-	20"
Number of treatments	-	9+1
Depth of sowing (loose soil)	-	1½ to 2 cm
Number of replicate	-	3

II. **Field experiment:**

Experiment design in field	-	RBD
Line to line distance	-	30cm
Number of treatments	-	9+1
Number of replicates	-	3
Cultivars	-	YS-66 & K-88

Both are grown in central India. (MP)

III. 1. First Irrigation - 20 days after sowing

2. Second Irrigation-30 days after sowing

3. Third Irrigation-50 days after sowing

Application of bio-fertilizers and seeds: Seeds were treated with fungicide i.e. thirum @ 3g/kg of seed then inoculated with *Azatobacter*, PSB culture, manually (a) 0.5 gm/kg, (b) 1.0 gm/kg, and (c) 1.5 gm/kg of seeds lot respectively. In third treatment *Azatobacter* culture were mixed with PSB thoroughly to make one single homogeneous lot. The mixing process should be done in shade. These bio-fertilizer were inoculated on two cultivars YS-66 and K-88 at the time of sowing except their respective controls, (un-inoculated)

RESULTS AND DISCUSSION: Table 1 and Table 2 show the plant height and number of branches per plant of the *Brassica campestris L*. treated with different biofertilizers and their combination.

Plant height at maturity: Plant height is an important index of plant growth which clearly indicates that the effect of different treatments on average plant height. In *Brassica campestris* L. cultivars YS-66 and K-88 a significant increase in plant height was observed in all the treatments of individual and combined treatment of *Azatobacter* and PSB during both years 2007-08 and 2008-09. *Azatobacter*, PSB and their combined treatments showed a prominent effect on plant height. Maximum plant height was observed under combined treatment of *Azatobacter* + PSB, 15 gm. + 15 gm, shows 160.3 ±1.9, 161.3±2.0 in cultivar YS-66 and 162.2 ± 1.8 , 162.2 ± 1.7 in cultivar K-88 against their control in both years. This might be due to dual effect of *Azatobacter*, first it fixes the nitrogen and secondly owing to synthesis of biologically active substances, similar result was reported by Sushila and Giri, (2000). While PSB increase the solubilization of phosphate and increase the availability of phosphorus, which help in proliferation of root system and increasing the nutrient absorption by increasing the absorbing surface increase in plant height Jakhar *et al.*, (2005) also reported similar findings in wheat. Badway and Amer, (1974) reported that inoculation of PSB enhanced the plant height in wheat and tomato Sutaliya and Singh, (2005) in maize, Govindan and Thirumirugan, (2005) in soybean and Purbey and Sen, (2007) in *Trigonella* observed that combination treatment of bio-fertilizers have prominent increase in plant height.

Number of Branches per plant: Number of branches per plant is another important parameter. Which increase the number of pods and finally yield. In all the treatments number of branches per plant significantly increase under *Azatobacter* 15 gm. treatment and combination of *Azatobacter* + PSB 5gm. + 5gm., 10gm.+ 10gm., 15gm. + 15gm. Maximum number of branches under *Azatobacter* + PSB 15 gm. + 15 gm. in YS-66 is 12.4±1.4, 12.3±1.6 against their control 6.2±1.2, 6.8±1.3 in two years respectively in K-88 maximum number of branches were 11.9±1.7, 12.3±1.8 against their controls 5.9±1.1, 6.1±1.3 in two years respectively *Azatobacter* + PSB combinations showed most prominent effect in two cultivars in this parameter. Micro-organisms secrete, organic acids which are responsible for solubilization of phosphate also produced amino acids, vitamins and growth promoting substances. *Azatobacter* and PSB provide more availability of nutrients particularly nitrogen and phsophorus due to which vegetative growth increase and number of branches increase similar result were obtained by Singh and Dutta, (2006) in rape seed and mustard.

Table 1: Effect of different doses of Biofertilizers Azatobacter, PSB and their combination on the Plant height (cm.) at maturity in two cultivars YS-66 and K-88 of *Brassica compestris L.*

S. No.	Biofertilizer	Treatments	Plant height at maturity (cm.)			
			Cultivar YS-66		Cultivar K-88	
			2007-08	2008-09	2007-08	2008-09
1	Azatobacter	5 gm.	145.3±1.9	146.2±1.8	147.3±1.7	148.2±1.8
2	Azatobacter	10 gm.	151.2±1.8	152.3±1.7	154.2±1.8	153.3±1.7
3	Azatobacter	15 gm.	154.3±1.7	155.2±1.6	155.2±1.7	154.2±1.8
4	PSB	5 gm.	144.2±1.9	145.3±1.8	146.2±1.7	146.0±1.6
5	PSB	10 gm.	150.2±1.7	151.3±1.8	151.2±1.7	152.7±1.6
6	PSB	15 gm.	152.2±1.9	153.3±1.7	152.3±1.2	154.2±1.2
7	Azatobacter+PSB	5 gm. + 5 gm.	153.2±1.7	154.7±1.1	154.2±1.1	153.3±1.1
8	Azatobacter+PSB	10 gm. + 10 gm.	156.6±1.9	157.3±1.2	158.3±2.0	159.4±1.9
9	Azatobacter+PSB	15 gm. + 15 gm.	160.3±1.9	161.3±2.0	162.2±1.8	162.2±1.7
10	Untreated	Control	144.2±3.2	146.3±4.2	145.2±3.9	146.3±3.2

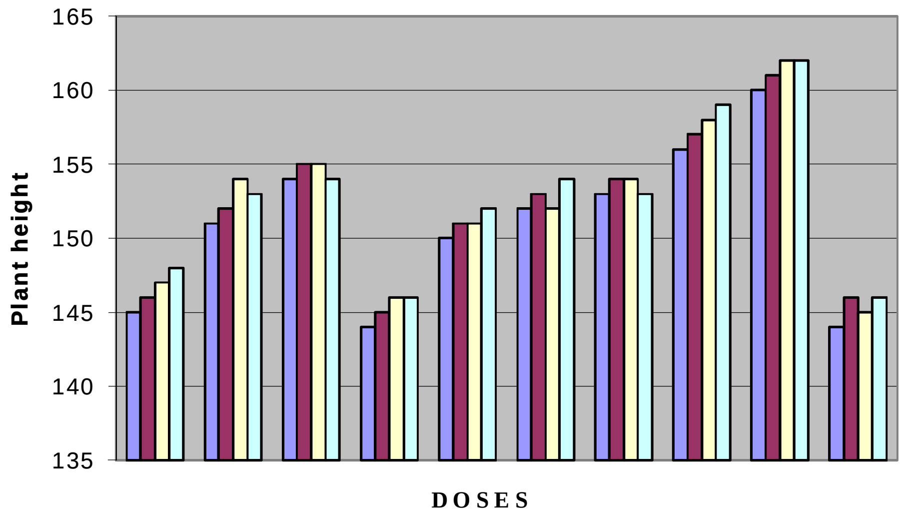
** LSD 0.05 =1.42; CV = 5.12*

Table 2: Effect of different doses of Biofertilizers Azatobacter, PSB and their combination on number of branches per plant in two cultivars YS-66 and K-88 of *Brassica campestris* L.

S. No.	Biofertilizer	Treatments	Number of branches per plant			
			Cultivar YS-66		Cultivar K-88	
			2007-08	2008-09	2007-08	2007-08
1	Azatobacter	5 gm.	6.9±1.4	6.7±1.3	6.8±1.1	6.7±1.2
2	Azatobacter	10 gm.	8.9±1.4	8.8±1.3	8.7±1.2	8.7±1.4
3	Azatobacter	15 gm.	10.2±1.3	10.3±1.7	11.1±1.4	11.00±1.3
4	PSB	5 gm.	6.8±1.3	6.6±1.2	6.7±1.2	6.5±1.1
5	PSB	10 gm.	8.1±1.4	8.0±1.1	8.3±1.1	8.1±1.4
6	PSB	15 gm.	9.7±1.2	9.69±1.3	9.7±1.3	9.6±1.1
7	Azatobacter+PSB	5 gm. + 5 gm.	9.24±1.3	9.1±1.2	9.4±1.1	9.3±1.1
8	Azatobacter+PSB	10 gm. + 10 gm.	10.2±1.4	10.1±1.3	10.2±1.2	10.2±1.3
9	Azatobacter+PSB	15 gm. + 15 gm.	12.4±1.4	12.3±1.6	11.9±1.7	12.3±1.8
10	Untreated	Control	6.2±1.2	6.8±1.3	5.9±1.1	6.1±1.3

**LSD 0.05 = 0.38; CV = 3.42*

Figure 1: Effect of different doses of Biofertilizers Azatobacter, PSB and their combination on the Plant height (cm.) at maturity in two cultivars YS-66 and K-88 of *Brassica compestris L.*



The report of 2n=2x=20 in the present species is in agreement with the previous reports of 2n=20 by Kaur and Singhal (2010), and Kaushla (2012) from India and by several authors from outside (see Table 1). Apart from the present species, chromosome number of the other species viz. *C. ruderalis* Janisch (2n=20, Krasnikov and Schaulo 1990; Stepanov and Muratova 1995) and *Cannabis sativa* subsp. *spontanea* (Czer.) Serebr. (2n=20, Murín and Svobodová. 1992; Kiehn *et al.* 2000) have also similar counts. The tetraploid (4x) meiotic chromosome count of 2n=40 in *Cannabis sativa* L. from cold desert area of Lahul-Spiti is reported for the first time. Cytologically all the diploid species have normal meiotic behavior with high (>90%) pollen viability. Presently the irregularities and disturbances in meiosis contributed to the degenerative processes of pollen development in tetraploid (4x) population and resulted in low (<70%) viability. The low pollen viability in (4x) population reflects the role of meiotic abnormalities especially laggards, bridges and stickiness of chromosomes as suggested in other species by Saggoo and Srivastava (2009); Srivastava (2012) and Kumar and Dwivedi (2013). Presence of heterogenous size pollen grains and high frequency of large sized pollen grains in (4x) population may be partially due to meiotic irregularities and partially due to cytomixis which are responsible for unequal chromatin distribution in these pollens. Similar observation and concept of cytomixis was made in other flowering plants by different workers (Saggoo and Srivastava 2009; Kalinka and Achrem 2010; Surender 2015; Rani 2014 Saggoo et al. 2011; Srivastava 2012; Kravets 2013). Now it is well known that cytomixis is a natural and normally genetically controlled phenomenon. But the influence of environmental factors (Jones and Newell 1948; Kurtz and Liverman 1958), nutrition (Bell 1959), ploidy (Gould 1957, Kapadia and Gould 1964) and geographical variation (Cain and Cain 1944) on pollen size and fertility in flowering plants could not be underestimated.

CONCLUSION: As per the results, it is clear that tetraploid (4x) meiotic chromosome count of 2n=40 in *Cannabis sativa* L. from cold desert area of Lahaul-Spiti is reported for the first time. Further, due to the absence of any significant morphological differences in between (2x) and (4x) populations, evolution towards 4x from 2x population under stress can be predictable, but in the absence of molecular and biochemical evidences it can only be hypothesized.

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