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Planting ratio and plant growth regulators affecting seed quality in CGMS based chilli (*Capsicum annuum*) hybrid

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ABSTRACT

In the present study, the seed production was carried out during 2016 and 2017 in the newly released cytoplasmic genetic male sterility (CGMS) based chilli (*Capsicum annuum*) hybrid. The objective of this study was to enhance the seed quality for newly released hybrid using given treatments in the field. The experiment was conducted at the Department of Seed Science and Technology, University of Agricultural Sciences, Raichur, Karnataka in the laboratory and field conditions. Plant growth regulators (PGRs) target specific receptor molecules in the plant tissue that can certainly transform plant metabolism. Similarly adapting a specific ratio of female and male while planting can check yield and quality constraints in fruit and seed production. Henceforth treatments were decided keeping in view these points. It was found that planting ratio (2:1 was best) and PGR spray (NAA @40 ppm best) significantly promoted the seed quality parameters, viz. germination (%), seedling dry weight (mg), shoot length (cm), seedling vigour index, dehydrogenase enzyme activity and lowered the electrical conductivity (dS/cm) as compared to other treatments. Also, the treatment combination P_2S_4 (2:1 + NAA @40 ppm) proved to be the best for these seed quality parameters. Thus, the parental lines in 2:1 ratio proved to be best for natural crossing followed by spraying at flower initiation stage with NAA @40 ppm as PGR to obtain higher seed quality in CGMS based chilli hybrid UARChH42 (JCH42). Hence it may be recommended in seed production technology of this hybrid.

Keywords: Chilli, CGMS based hybrid, Plant growth regulators, Planting ratio, Seed quality

Chilli (*Capsicum annuum* L.) is an important spice as well as vegetable in world fetching higher price as a commercial crop. The name *Capsicum* is derived from The Greek word 'Kapsimo' which means 'to bite'. This member of nightshade family solanaceae, is mainly a group of tropical as well as temperate plants belonging to 75 genera and 2000 species (Himangini *et al*. 2021). In the year 1885 chilli was carried and introduced by Portuguese from Brazil to India (Bassett 1986). It is cultivated on an area of 364 thousand hectares in India with a production of 3720 thousand metric tonnes 2018-19 (Anonymous 2019). Chilli is among top 10 exported spices from India. Out of the major 5 species of chilli which are cultivated amongst the 20–30 species of *Capsicum* the Raja Mirch or Bhut Jolokia commonly known as the Nagaking chili (*C. chinense* Jacq.) from Nagaland state of India holds the Guinness book of world records, 2000 for world's hottest chilli (Ananthan *et al*. 2014).

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In chilli there is about 40–50% fruit set only (Himangini *et al.* 2022) and the most popular technique of hybrid seed production is emasculation and pollination [Thakur (b) *et al*. 2020 and Thakur *et al*. 2021]. In the present research CGMS is employed to produce hybrid seed that can also reduce the cost of skilled labourers needed for emasculation and pollination thus, further reduces hybrid seed cost about 50% (Aulakh 2016). For the implementation of CGMS the male and female parents identified through molecular test (Thakur *et al.* 2022) were sown in different ratios. Afterwards hybrid fruits were harvested from the female parent. The 2:1 planting ratio produced higher seed yield in chilli hybrid as reported by Khurana *et al.* (2002) and Thakur (a) *et al*. (2020). PGRs, viz. Gibberellic acid (GA_3), Naphthalene Acetic Acid (NAA) and Cytokinins are considered as new generation plant growth stimulating agrochemicals after fertilizers, pesticides and herbicides. It modifies the growth, sex ratios and yield contributing characters of plant (Shantappa *et al.* 2007). The present investigation was conducted to produce hybrid seeds of chilli hybrid using PGRs and different planting ratios in amalgamation with CGMS.

MATERIAL AND METHODS

Present study was carried out at the Department of

Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka during rainy (*kharif*) season 2016 and 2017. The seeds of A line (JNA-1) and R line (BVC-42) were obtained from the department of genetics and plant breeding, UAS Raichur and were pre-treated with Imidacloprid 0.4%. Seeds were sown separately in pro-trays inside nursery under shade net during June 2016 and 2017. About 45 days-old well developed seedlings were transplanted in the main field at spacing of 90 cm (row-to-row) and 60 cm (plant-to-plant). These seedlings were transplanted in different female: male ratio, viz. 1:1, 2:1, 3:1 and 3:2 and enough plants were sown in the single row of each ratio to meet the desired plant population. Also, the chemical regulating plant growth serves to ameliorate the physiological characteristics of plant. Hence the treatments were decided as foliar sprays of different plant growth regulators (PGR), viz. control (no chemical spray), 2, 4-D (2,4-Dichlorophenoxyacetic acid) @2 ppm, GA_3 (Gibberellic acid) @50 ppm and NAA (Naphthaleneacetic acid) @40 ppm. Plants of A line/ female parent/ seed parent were sprayed with these PGRs at initial stages of flowering and then at 30 days interval. The experimental design used was Factorial with 16 treatment combinations (4 planting ratios × 4 PGR sprays). It was laid out in Factorial Randomized Complete Block Design (FRCBD) in the field and Factorial Completely Randomized Design (FCRD) in the laboratory. For each treatment seed quality parameters were recorded. As per ISTA (International Seed Testing Association, Zurich) guidelines (Anonymous 2013) 400 seeds were taken and put for germination (between paper) in four replications to conduct the test, viz. germination (%), shoot length (cm), root length (cm) and seedling dry weight (mg). Further with its seedling vigour index was evaluated using the procedure of Abdul-Baki and Anderson (1973). The chemical composition of seeds tell much about the quality of seed and its performance in field in terms of radical protrusion, plumule growth and field emergence. Hence, seed biochemical parameters were also recorded in addition to seed physiological quality. These were electrical conductivity (dS/cm i.e. deci Siemens per centimeter), α-amylase (alpha amylase) enzyme activity (diameter of halos) and dehydrogenase enzyme activity (OD value i.e. Optical Density) (Kittock and Law 1968). The data were recorded in three (in FRCBD) and four (in FCRD) replications respectively. Statistical analysis was carried out for each observed character using MS-Excel with formulas incorporated. The mean values of data for FRCBD and FCRD were subjected to analysis of variance as per the design of experiment as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The analysis of variance results revealed that planting of the parent lines in P_2 (2:1) planting ratio resulted in highest seed physiological and biochemical parameters. Fruits as well as seeds obtained from this treatment showed overall superiority over other treatment and their combinations. This might be attributed due to higher reserve food found in the seeds of these treatments which helped them to show higher quality. In 2:1 planting ratio the amount of pollen grains available was ample for proper fruit and seed set, seed formation and development (Table 1). Maximum germination (80.67 and 83.92%), shoot length (8.13 and 10.00 cm), seedling dry weight (2.74 and 3.17 mg), seedling vigour index (1606 and 1951), lower electrical conductivity (0.098 and 0.097 dS/cm) and higher dehydrogenase enzyme activity (0.189 and 0.198) and α-amylase enzyme activity (12.21 and 13.00) was recorded in this planting ratio during 2016 and 2017 respectively as compared to other treatments. Whereas, planting ratio P_4 (3: 2) recorded lowest values of seed quality characters which may be due to deposition of inadequate viable pollens on the stigma of A line. As the planting ratio increased less male plants were available per female plants in the field. It lowered the seed set that render physiologically and biochemically unfit seeds as a consequence of under developed embryo. These might have reduced the fruit set as well as seed production. Similar results were obtained by Kumar *et al.* (2008) in tomato who reported increased seed quality parameters in 3:1, 4:1 and 5:1 ratio attributed due to higher reserve found in the seeds of these treatments and also, by Patil *et al.* (2008) in brinjal and Gowda *et al.* (2017) in okra. In ridge gourd Lambat *et al.* (2015) reported better seed quality when crop was sprayed with NAA @50 ppm and in coriander Pranay *et al.* (2019) reported better seed quality parameters in treatment GA_3 @100 ppm. The electrical conductivity (EC) of a seed presents the integrity of its coat's cell membrane. Higher is the EC value of the seed soak; higher are the chances that cell membrane is damaged. Henceforth, it was found that there was significant variation in the values of electrical conductivity in given treatment 2:1 ratio. It may be due to the anatomical structure, membrane permeability and composition of seed coat which reduced the EC value under this treatment because of more intact and sound seed coat cell membrane.

PGRs applied in optimum doses to plants at flowering stage triggered fruit growth and seed development. In case of the application of NAA @40 ppm it is ascribed to provide the adequate supply of food reserves to resume embryo growth. This also helps in synthesis of hydrolytic enzymes which are secreted and act on starchy endosperm, this in turn affects physiology of seed. It resulted in higher germination (78.00 and 81.08%), shoot length (8.08 and 9.86 cm), seedling dry weight (2.67 and 3.03 mg) and ultimately the higher seedling vigour index (1552 and 1872) in 2016 and 2017 respectively (Table 1).

Similar results were obtained by Wang *et al.* (2019) who reported 2.5–6.3% and 8.9–19.8% increase in germination percentage and vigour index respectively from GA_3 (30 g/ hm^2) application after anthesis in rice. Seed biochemical parameter, viz. higher dehydrogenase enzyme activity (0.203 and 0.212 OD value), α-amylase enzyme activity (12.71 and 15.70) and lower electrical conductivity (0.096 and 0.093 dS/cm), was also observed in NAA @40 ppm

Table 1 Effect of planting ratio and foliar spray of plant growth regulators on seed quality parameters in chilli hybrid UARChH42 (JCH42)

Treatment	Germination (%)*		Shoot length (cm)		Seedling dry weight (mg)		Seedling vigour index		EC (dS/cm)		DAA (OD value)		AAEA (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
P_1	72.00^{a} (58.12)	74.67^{a} (59.89)	7.01^{a}	8.80^{a}	2.40	2.87	1199^{a}	1519	0.114^{a}	0.110^{a}	0.166	0.172	9.02	11.56^{b}
P_2	80.67 (64.05)	83.92 (66.55)	8.13	10.00	2.74	3.17	1606	1951	0.098	0.097	0.189	0.198	12.21	13.00 ^{a}
P_3	70.42^{a} (57.15)	73.58^{a} (59.23)	7.12^{a}	7.98^{b}	2.26 ^{a}	2.45 ^{a}	1191^{a}	1380^{a}	0.113^{a}	0.108 ^{a}	0.146	0.153	9.00	11.77^{ab}
P_4	70.42^{a} (57.07)	73.25^{a} (58.89)	6.93^{a}	8.23^{ab}	2.18 ^{a}	2.46 ^{a}	1165^{a}	1399^{a}	0.122	0.117	0.131	0.136	7.11	8.15
S.Em±	0.50	0.62	0.083	0.151	0.027	0.062	13.16	26.57	0.001	0.001	0.002	0.003	0.32	0.37
CD @1%	1.92	2.39	0.320	0.584	0.106	0.240	50.98	102.88	0.004	0.003	0.010	0.010	1.25	1.43
S_1	71.75 (57.94)	74.25 (59.58)	6.89 ^{a}	8.27^{a}	2.29	2.66 ^{a}	1129^{a}	1367 ^{a}	0.116	0.111	0.140	0.145	8.47	10.49 ^{a}
S_2	67.75 (55.48)	70.42 (57.15)	6.81^{a}	7.83^{a}	2.13	2.40	1086^{a}	1299 ^{a}	0.130	0.124	0.117	0.119	5.85	6.07
S_3	76.00 (60.80)	79.67^{a} (63.39)	7.40	9.05	2.49	2.85 ^{a}	1393	1712	0.106	0.104	0.172	0.181	10.30	12.22 ^{a}
S_4	78.00 (62.16)	81.08^{a} (64.42)	8.08	9.86	2.67	3.03	1552	1872	0.096	0.093	0.203	0.212	12.71	15.70
S.Em±	0.50	0.62	0.083	0.151	0.027	0.062	13.16	26.57	0.001	0.001	0.002	0.003	0.32	0.37
CD @1%	1.92	2.39	0.320	0.584	0.106	0.240	50.98	102.88	0.004	0.003	0.010	0.010	1.25	2.87
P_1S_1	70.33^{cd} (57.00)	71.33^{d} (57.63)	6.67^{c}	8.97^{b}	2.27	2.92	1023^{c}	1387^{c}	0.115^{d}	0.110^{c}	0.147^{d}	0.152^{ef}	8.06	9.43
P_1S_2	66.33^{e} (54.54)	69.00^{d} (56.18)	6.59^{c}	7.81^{c}	2.11	2.44	1016^{cd}	1256^{d}	0.136^{ef}	0.127^{g}	0.132^{ef}	0.136^{f}	5.45	6.78
P_1S_3	74.33^{b} (59.57)	77.67^{b} (61.81)	7.12^{b}	8.92^{b}	2.47	2.90	1307^{b}	1646^{ab}	0.107^{bc}	0.106^{b}	0.179^{bc}	0.187^{cd}	9.31	12.69
P_1S_4	77.00^{b} (61.35)	80.67^{b} (63.93)	7.66^{ab}	9.51^{ab}	2.74	3.23	1450^{a}	1789^{a}	0.100^{ab}	0.097^{a}	0.207^{a}	0.213^{ab}	13.25	17.32
P_2S_1	77.67^{b} (61.81)	80.67^{b} (63.94)	7.57^{b}	8.80^{b}	2.63	2.92	1306^{b}	1503^{bc}	0.105^{b}	0.106^{b}	0.166^{c}	0.168 ^{de}	12.31	13.58
P_2S_2	76.00^{b} (60.68)	79.00^{b} (62.73)	7.32^{b}	8.52^{b}	2.41	2.81	1290^{b}	1571^{b}	0.116^{d}	0.117^{ef}	0.130^{e}	0.134^{fg}	8.39	6.52
P_2S_3	83.67^{a} (66.23)	87.33^{a} (69.19)	8.25^{a}	10.63^{a}	2.89	3.40	1763	2206	0.094^{a}	0.090	0.213^{a}	0.227^{a}	12.85 ^{a}	14.71
P_2S_4	85.33^{a} (67.48)	88.67^{a} (70.33)	9.37	12.05	3.03	3.56	2063	2525	0.079	0.076	0.246	0.263	15.27 ^{a}	17.19
P_3S_1	70.00^{d} (56.17)	73.00^{c} (58.70)	6.68^{c}	7.51^{cd}	2.21	2.44	1093^{c}	1261^{d}	0.117^{d}	0.108^{c}	0.131^{e}	0.136^{f}	7.50	11.71
P_3S_2	61.33 (51.55)	63.67 (52.95)	6.62^{c}	6.80^{d}	2.03	2.14	963^{d}	1043	0.126^{e}	0.117^{e}	0.108^{gh}	0.108^{hi}	4.09	7.20
P_3S_3	74.00^{bc} (59.35)	79.00^{b} (62.76)	7.19^{b}	8.42^{bc}	2.34	2.56	1294^{b}	1569^{b}	0.107^{bc}	0.107^{bc}	0.149^{d}	0.163^{e}	11.14	12.23
P_3S_4	76.33^{b} (60.90)	78.67^{b} (62.51)	7.99^{a}	9.17^{b}	2.46	2.65	1414^{a}	1647^{a}	0.100^{ab}	0.101^{ab}	0.196^{ab}	0.202^{bc}	13.28 ^{a}	15.94
P_4S_1	69.00^{d} (56.17)	72.00^{cd} (58.07)	6.65^{c}	7.81^{c}	2.05	2.37	1094^{c}	1315^{cd}	0.127^{e}	0.121^{fg}	0.116^{fg}	0.125^{gh}	6.02	7.22
P_4S_2	67.33^{de} (55.15)	70.00^{d} (56.80)	6.71^{c}	8.17^{c}	1.99	2.23	1073^{c}	1324^{c}	0.143^{f}	0.135	0.096^{h}	0.099^{i}	5.45	3.76
P_4S_3	72.00^{c} (58.05)	74.67^{c} (59.80)	7.03^{bc}	8.23^{c}	2.25	2.56	1209^{b}	1428^{c}	0.114 ^{cd}	0.112^{ce}	0.146^{de}	0.147^{f}	7.91	9.26
P_4S_4	73.33^{c} (58.92)	76.33^{bc} (60.90)	7.31^{b}	8.73^{b}	2.43	2.68	1282^{b}	1529^{b}	0.106^{b}	0.100^{a}	0.164^{cd}	0.171^{d}	9.04	12.35
Mean	73.38	76.35	7.30	8.75	2.39	2.74	1290	1562	0.112	0.108	0.158	0.164	9.333	11.119
S.Em±	0.99	1.23	0.165	0.302	0.055	0.124	26.33	53.13	0.002	0.002	0.005	0.005	0.64	0.74
CD @1%	3.85	4.78	0.641	1.168	NS	NS	101.97	205.77	0.007	0.006	0.019	0.020	2.49	NS

Values followed by same alphabets are on par with one another. *Figures in parenthesis represent arcsine transformation.

P_1, 1:1; P_2, 2:1; P_3, 3:1; P_4, 3:2; S_1, no chemical spray; S_2, 2, 4-D @2 ppm; S_3, GA_3 @50 ppm; S_4, NAA @40 ppm; P_1S_1, 1:1 + no chemical spray; P_1S_2, 1:1 + 2, 4-D @2 ppm; P_1S_3, 1:1 + GA_3 @50 ppm; P_1S_4, 1:1 + NAA @40 ppm; P_2S_1, 2:1 + no chemical spray; P_2S_2, 2:1 + 2, 4-D @2 ppm; P_2S_3, 2:1 + GA_3 @50 ppm; P_2S_4, 2:1 + NAA @40 ppm; P_3S_1, 3:1 + no chemical spray; P_3S_2, 3:1 + 2, 4-D @2 ppm; P_3S_3, 3:1 + GA_3 @50 ppm; P_3S_4, 3:1 + NAA @40 ppm; P_4S_1, 3:2 + no chemical spray; P_4S_2, 3:2 + 2, 4-D @2 ppm; P_4S_3, 3:2 + GA_3 @50 ppm; P_4S_4, 3:2 + NAA @40 ppm. EC, Electrical Conductivity; DAA, Dehydrogenase enzyme activity; AAEA, Alpha amylase enzyme activity (Diameter in mm).

spray in 2016 and 2017 respectively. Lowest values of seed quality parameters were observed in S_2 (2, 4-D @2 ppm spray) which did not affect the quality parameters very well. These outcomes are in conformity with the findings of Arvindkumar *et al.* (2012) in bitter gourd seeds wherein NAA 50 ppm showed higher seed quality because food reserves are supplied adequately to resume embryo growth and synthesis of hydrolytic enzymes that alters the physiology of seed germination, establishment of seedling and ultimately the vigour index. Similar results were also reported by Kallihal (2012) in pigeon pea and in okra by Shahid *et al.* (2013).

The treatment combination of 2:1 planting ratio and NAA @40 ppm as PGR spray showed higher values of seed quality and biochemical parameters. In general, a planting ratio of 2:1 for parental lines followed by natural crossing and foliar spray of NAA @40 ppm as plant growth regulator can be recommended for obtaining higher seed quality parameters (up to 8% more germination than no spray) in newly released chilli hybrid UARChH42 (JCH42).

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