



Exogenous application of GA₃ influences morphological traits and developmental transitions in *Or* gene introgressed Indian cauliflower (*Brassica oleracea* var. *botrytis*)

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ABSTRACT

The present study was carried out during the winter (*rabi*) season of 2021–22 and 2022–23 at ICAR-Indian Agricultural Research Institute, New Delhi aimed to study the impact of GA₃ spray on morphological traits and developmental transitions in *Or* gene introgressed CF_{Or} (PKVA-1_{HM}) and white CF_{WT} (DC 18–19) genotypes of Indian cauliflower (*Brassica oleracea* var. *botrytis* L). Total of 24 treatment combinations comprising four GA₃ concentrations, viz. G₀, Control; G₁, 250 ppm; G₂, 500 ppm and G₃, 1000 ppm; two genotypes, CF_{Or} (homozygous orange) and CF_{WT} (white); three applications at different plant stages, viz. S₁, Vegetative stage (VS); S₂, VS + curd initiation stage (CIS) and S₃, VS + CIS + full curd stage (FCS) in factorial randomised block design (Factorial- RBD) with three replications. Observations were recorded for six morphological traits and five developmental transitions. Significant differences were found for all three factors, viz. GA₃ concentration, genotypes and plant stages. A spray of 500 ppm GA₃ on CF_{Or} and CF_{WT} genotype at VS + CIS stage resulted in superior performance in morphological traits and developmental transitions. It resulted maximum stalk length (69.33 cm) in CF_{Or}, however, it was statistically similar to 250 ppm GA₃ spray at VS + CIS stage in CF_{WT} (69.83cm). Both levels resulted in synchronization in flowering time of CF_{Or} (153 days) and CF_{WT} (153.5 days). The findings highlight the role of GA₃ application in CF_{Or} and CF_{WT} genotypes for improving morphological traits and synchronize flowering for hybrid seed production.

Keywords: Developmental transitions, GA₃ spray, Orange cauliflower, Synchronization in flowering

Cauliflower (*Brassica oleracea* var. *botrytis* L.) a key member of the family Brassicaceae, is an important cole crop preferred all over the world for its delicious taste, flavour and purported anti-cancerous glucosinolates and other essential minerals and vitamins (Singh and Kalia 2021). In India, it is grown over 4,58,000 ha area yielding 8.84 million tonnes annually (NHB 2020). The edible part of cauliflower is curd, consisting of proliferating, arrested inflorescence, and floral meristems. White curds are popular but other colours also exist in nature, namely orange (carotenoids), purple (anthocyanins) and green (chlorophyll) (Kalia *et al.* 2023). *Or* gene mutation is a spontaneous, semi-dominant gene mutation (Crisp *et al.* 1975). *Or* gene was introgressed into white colour elite inbred line of Indian cauliflower through marker-assisted backcross breeding at ICAR-Indian Agricultural Research Institute, New Delhi, enhancing the β -carotene content up to 12 ppm (Kalia *et al.* 2018, Kalia and Singh 2020). In addition to conferring high levels of β -carotene accumulation, *Or* gene has pleiotropic effects on

plant growth and development including enhanced petiole elongation, small curds formation and late flowering in *Or* homozygous mutant (Crisp *et al.* 1975, Li *et al.* 2001).

Gibberellin (GA) has significant influence on plant growth, development and onset of flowering (Hedden and Sponsel 2015). It promotes stem elongation and transition from vegetative to the reproductive phase (Taiz and Zeiger 2002). It is known to stimulate cell elongation, leaf area expansion and overcome dwarfism (Ramesh and Kumar 2006) and also improves stress tolerance in crop plants (Nagar *et al.* 2022). In *Brassica oleracea*, stems elongation at bolting stage is regulated by gibberellin (Mandel *et al.* 1992). Duclos and Bjorkman (2015) reported the role of GA in reproductive transitions and stem elongation in broccoli. GA₃ application also stimulated bolting, flowering and seed production in white cauliflower (Prodhan *et al.* 2022). Early curd maturity was reported in mid group white curd cauliflower with the spray of GA₃ (200 ppm) by Sonam *et al.* (2020) and in European type cauliflower with GA₃ @800 ppm by Yacoub and Abbas (2012).

The '*Or*' gene in homozygous lines severely impact plant growth, delays flowering and seed setting which

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complicates hybrid seed production due to non-synchronized flowering in parental lines (Crisp *et al.* 1975). Since, GA₃ has shown promise for earliness in transitions without negative effect on morphological traits, suggesting its potential for mitigating the delays induced by the 'Or' gene in orange cauliflower. Therefore, the present study aimed to determine the appropriate concentration and application stage of GA₃ in white and orange (*Or* gene introgressed) genotypes of Indian cauliflower to synchronize their flowering time to ease crossing.

MATERIALS AND METHODS

The present study was carried out during winter (*rabi*) season of 2021–22 and 2022–23 at ICAR-Indian Agricultural Research Institute, New Delhi. The experimental site was characterized by a semi-arid, sub-tropical climate with sandy loam soils. The experiment followed a factorial randomised block design (Factorial-RBD) with three replications. Each plot had 20 plants grown in a two-row system. Seeds treated with Bavistin (@2 g/kg seed) were sown in nursery beds and one-month-old seedlings were transplanted at a spacing of 45 cm × 45 cm in open field condition. Recommended crop practices were followed as per Singh and Sharma (2003).

The 24 treatments included four GA₃ concentrations i.e. G₀, Control; G₁, 250 ppm; G₂, 500 ppm and G₃, 1000 ppm; two genotypes, viz. CF_{Or} (homozygous orange derived from Pusa Kesari Vit. A-1 (PKVA-1_{HM}) and CF_{WT} (white curd, DC 18–19) and three growth stages for application viz., S₁, Vegetative Stage (VS); S₂, VS + curd initiation stage (CIS) and S₃, VS + CIS + full curd stage (FCS) (Table 1). GA₃ was applied at noon using a hand sprayer. Observations for plant height (cm), plant spread (cm), leaf length (cm), leaf width (cm), number of leaves/plants, curd height (cm), stalk length (cm), days to curd initiation [days after transplanting (DAT)], curd maturity (DAT), bolting (DAT), flower bud formation (DAT), and 50% flowering (DAT) were recorded on 15 plants/replication.

Table 1 Experimental design of the GA₃ spray in cauliflower

Treatment		Developmental stages		
		Vegetative stage	Curd initiation stage	Full curd stage
G ₀ (Control)		-	-	-
G ₁ (250 ppm)	S ₁	Y	-	-
	S ₂	Y	Y	-
	S ₃	Y	Y	Y
G ₂ (500 ppm)	S ₁	Y	-	-
	S ₂	Y	Y	-
	S ₃	Y	Y	Y
G ₃ (1000 ppm)	S ₁	Y	-	-
	S ₂	Y	Y	-
	S ₃	Y	Y	Y

Y, GA₃ spray; -, No spray; S₁, Vegetative stage; S₂, Vegetative stage + Curd initiation stage; S₃, Vegetative stage + Curd initiation stage + Full curd stage.

Statistical analysis: Analysis of variance (ANOVA) was performed for three-factor randomised complete block design (RCBD) to assess the effect of genotype, GA₃ concentration and stage of application and their interactions for morphological traits and developmental transition stages across both years. Pooled data were analysed using RStudio's 'doebioresearch' package, with significant differences identified by Duncan's multiple range test (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) demonstrated highly significant mean sum of squares for all 3 factors, viz. stage of application, genotypes and GA₃ concentrations for most of the observed morphological traits and developmental transitions across both years (2021–22 and 2022–23) as well as pooled data of *Or* gene introgressed lines of Indian cauliflower (Table 2). The effect of years also showed significant differences for all the morphological traits and developmental transitions except leaf width; however, we presented here pooled data for three factors only.

Effect on morphological traits: Leaf length, leaf width, number of leaves/plants, plant height, plant spread and curd height are significantly influenced by the application stage, genotypes and GA₃ concentration (Table 3). Maximum leaf length (52.9 cm) and number of leaves (21.8) were obtained from GA₃ application at VS + CIS stage which was statistically at par with VS + CIS + FCS stage followed by VS stage in the pooled data. Leaf width was observed to be the highest from GA₃ spray at VS + CIS stage which was statistically similar to GA₃ spray at VS stage and significantly higher than VS + CIS + FCS stage. Maximum plant height (62.7 cm), plant spread (68.9 cm) and curd height (32.9 cm) was achieved by GA₃ spray at VS + CIS stage which was significantly higher than spray at VS + CIS + FCS stage and VS stage. Similarly, longest stalk length (66.4 cm) was observed with GA₃ applied at VS + CIS stage followed by VS + CIS + FCS stage and VS stage alone. These differences in growth parameters were resulted by modifications in physiological processes induced by varying GA₃ application stages. The present results are aligning with the findings of Kou *et al.* (2021) and Nakajima *et al.* (2023).

Both CF_{Or} and CF_{WT} exhibited significant differences in response to GA₃ application at different developmental stages (Table 3). CF_{WT} have significantly higher values of leaf length (56.7 cm), leaf width (22.0 cm), number of leaves (23.3), plant height (70.6 cm), plant spread (72.6 cm), curd height (42.1 cm) and stalk length (70.3 cm) compared to CF_{Or}. Irrespective of the stage of application, the response to GA₃ application was significantly higher in CF_{Or} genotype as compared to CF_{WT}. CF_{Or} genotype showed greater response for the changes in morphological traits than CF_{WT} genotype with GA₃ application over the control. The *Or* gene affect the morphological and developmental changes in cauliflower plant and require exogenous application of growth hormones. Unlike homozygous dominant *Or* gene, the white cauliflower does not have penalty on morphology

Table 2 ANOVA for effect of plant stages, genotypes and GA₃ concentration on *Or* gene introgression lines of cauliflower (2 years pooled data)

Source of variation	DF	Leaf length (cm)	Leaf width (cm)	Leaf number/plant	Plant height (cm)	Plant spread (cm)	Curd height (cm)	Stalk length (cm)	Days to initiation (DAT)	Days to curd stage (DAT)	Days to bolting (DAT)	Days to flower bud formation (DAT)	Day to 50% flowering (DAT)
Replications	2	2.11	0.39	0.72	0.5	0.13	1.4	1.8	1.45	2.26	1.01	1.6	2.3
Plant stage (A)	2	36.56***	8.72***	9.60*	25.2***	124.44***	68.3***	255.8***	2.51	28.18***	130.23***	161.9***	243.9***
Genotypes (B)	1	1581.00***	834.36***	348.92***	5759.3***	2928.93***	8492.1***	3321.1***	1810.01***	2738.00***	2958.09***	3472.2***	4050.0***
GA ₃ concentration (C)	3	174.16***	16.88***	38.98***	389.9***	99.51***	109.9***	1199.7***	579.36***	1057.91***	1792.61***	2047.8***	2563.0***
A × B	2	12.40***	5.01**	12.06**	4.5*	27.81***	10.6***	22.2***	1.07	10.67**	12.75***	12.0***	5.2*
A × C	6	2.98	0.35	1.75	2.6*	9.97***	5.0**	6.8***	2.17*	3.61*	11.15***	12.5***	19.5***
B × C	3	26.04	0.11	5.38*	3.8*	20.96***	3.9*	33.9***	112.88**	175.60***	189.84***	198.90***	181.8***
A × B × C	6	3.01***	0.22	2.23	0.5	1.35	1.7	4.9**	2.02	2.63	1.10	2.1	2.8*
Error	46	1.32	0.88	1.90	0.9	2.14	1.3	1.2	0.91	1.41	1.32	1.1	1.2

DF, Degree of freedom; DAT, Days after transplanting. *Significant at 5%; **Significant at 1%; ***Significant at 0.1% when tested against MSS due to error.

and developmental processes. Thus, the response of exogenous application of GA₃ was more profound in CF_{Or} genotype. Further, gibberellins, synthesized in plastids and translocated into cytosol in biological active forms, regulate diverse morphological processes such as stem growth, flowering time and vegetative and reproductive development (Kasahara *et al.* 2002, Sun and Gubler 2004). Notably, *Or* gene is responsible for conversion of plastid to chromoplast, may affect the fate of exogenous GA₃ and ultimately its response to morphological traits and days to developmental transitions. CF_{WT} naturally outperforms CF_{Or} in morphological traits (Crisp *et al.* 1975) which also further improved with GA₃ applications. Wang *et al.* (2022) reported that the *Or* gene in *Arabidopsis thaliana* delayed-flowering by negatively regulating the expression of the flowering locus t (*FT*) and suppressor of overexpression of constans 1 (*SOC1*) genes of flowering pathway.

The effect of different GA₃ concentrations was significant for all observed morphological traits (Table 3). The longest leaf length (55.3 cm) and plant height (66.3 cm) were obtained with 500 ppm GA₃ which was significantly higher than 1000 ppm GA₃. The 500 ppm GA₃ spray also resulted in significantly wider leaves, more number of leaves/plant, plant spread and stalk length (19.7 cm, 22.6, 68.9 cm and 71.0 cm, respectively). This optimal dose of gibberellic acid likely promotes vegetative growth by enhancing cell elongation and cell division in sub-apical meristem (Kaur and Mal 2018).

Interaction between stage of application and genotypes was found significant for all the observed morphological traits (Fig. 1a). The longest leaf length and plant height were recorded in CF_{WT} with GA₃ spray at VS + CIS stage (57.4 cm and 71.9 cm) followed by VS + CIS + FCS stage (56.7 cm and 70.7 cm) and VS stage (56.1 cm and 69.1 cm). The maximum leaf width was observed in CF_{WT} when sprayed at VS followed by VS + CIS stage and VS + CIS + FCS stage. The highest plant spread was also noticed in CF_{WT} (76.5 cm) at VS + CIS stage which was 19.8% more than CF_{Or}. Irrespective of the application stage, the highest number of leaves/plant was observed in CF_{WT} followed by CF_{Or} at VS + CIS stage which was statistically similar to VS + CIS + FCS in CF_{Or}. Maximum curd height was observed in CF_{WT} at VS + CIS stage statistically similar to VS + CIS + FCS stage. A similar trend of hierarchy was followed in CF_{Or} although the minimum leaf length (45.1 cm), leaf width (15.3 cm), plant height (52.2 cm), plant spread (52.2 cm), number of leaves (17.8), curd height (18.4 cm) and stalk length (52.1 cm) were recorded in CF_{Or} when spray was done at VS stage. The increase in morphological traits could be due to increased chloroplast by GA application (Jiang *et al.* 2012).

The interaction between application stage and GA₃ concentration was significant for plant height, plant spread, curd height and stalk length (Fig. 1b). The maximum plant height (67.7 cm), plant spread (71.1 cm), curd height (35.5 cm) and stalk length (75.2 cm) were observed when 500 ppm was applied at VS + CIS stage which was statistically similar to 250 ppm at VS + CIS stage for plant spread

Table 3 Effect of time of application, genotypes and GA₃ concentration on morphological traits and developmental transitions on *Or* gene introgression lines of cauliflower (2 years pooled data)

Treatment	Leaf length (cm)	Leaf width (cm)	Leaf number/plant	Plant height (cm)	Plant spread (cm)	Curd height (cm)	Stalk length (cm)	Days to curd initiation (DAT)	Days to full curd stage (DAT)	Days to bolting (DAT)	Days to flower bud formation (DAT)	Day to 50% flowering (DAT)
Stage of application												
S ₁ = VS	50.6±6.1 ^b	19.0±3.8 ^a	20.5±3.2 ^b	60.7±9.3 ^c	64.7±6.5 ^b	29.6±11.3 ^c	60.0±10.5 ^c	95.0±7.49 ^a	114.2±9.83 ^a	137.3±11.2 ^a	148.0±11.9 ^a	166.7±12.7 ^a
S ₂ = VS + CIS	52.9±5.6 ^a	19.0±3.1 ^a	21.8±2.5 ^a	62.7±10.3 ^a	68.9±8.3 ^a	32.9±10.9 ^a	66.4±10.1 ^a	94.4±7.27 ^b	112.0±9.24 ^c	132.6±11.3 ^c	142.8±12.1 ^c	160.3±13.5 ^c
S ₃ = VS + CIS + FCS	52.6±5.3 ^a	17.9±3.9 ^b	21.0±3.0 ^{ab}	61.5±10.0 ^b	65.3±5.9 ^b	31.2±11.6 ^b	64.0±9.4 ^b	94.7±7.48 ^{ab}	113.1±9.57 ^b	135.3±11.2 ^b	145.6±12.0 ^b	163.4±13.3 ^b
SEm±	0.2	0.19	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CD (at 5%)	0.7	0.5	0.8	0.6	0.8	0.7	0.6	NS	0.7	0.7	0.6	0.6
Genotype												
V ₁ = CF _{For}	47.3±4.2 ^b	15.2±1.3 ^b	18.9±2.4 ^b	52.7±4.6 ^b	59.9±3.5 ^b	20.4±2.9 ^b	56.7±8.3 ^b	99.7±7.16 ^a	119.3±9.6 ^a	141.5±11.8 ^a	152.4±12.5 ^a	171.0±13.5 ^a
V ₂ = CF _{WT}	56.7±2.1 ^a	22.0±1.3 ^a	23.3±1.5 ^a	70.6±3.9 ^a	72.6±3.3 ^a	42.1±2.9 ^a	70.3±7.3 ^a	89.7±2.92 ^b	106.9±4.1 ^b	128.6±6.1 ^b	138.5±6.7 ^b	156.0±8.1 ^b
SEm±	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
CD (at 5%)	0.5	0.4	0.7	0.5	0.7	0.5	0.5	0.5	0.6	0.5	0.5	0.5
GA ₃ concentration												
G ₀ = Control	48.0±6.4 ^d	17.4±3.5 ^c	19.1±3.1 ^c	55.6±9.5 ^d	63.2±8.1 ^c	27.9±10.5 ^d	52.8±8.5 ^d	101.6±7.82 ^a	122.6±10.0 ^a	147.8±10.3 ^a	159.1±11.0 ^a	179.0±11.3 ^a
G ₁ = 250 ppm	51.4±5.3 ^c	18.9±3.6 ^b	21.2±2.6 ^b	60.6±9.4 ^c	66.8±7.1 ^b	30.9±10.7 ^c	61.6±5.8 ^c	96.9±6.63 ^b	115.8±7.8 ^b	138.0±8.5 ^b	148.3±9.1 ^b	166.2±9.9 ^b
G ₂ = 500 ppm	55.3±3.7 ^a	19.7±3.6 ^a	22.6±1.9 ^a	66.3±8.5 ^a	68.9±5.7 ^a	33.6±11.5 ^a	71.0±8.0 ^a	89.0±2.72 ^d	105.5±3.4 ^d	125.4±3.9 ^d	135.0±4.4 ^d	151.9±5.3 ^d
G ₃ = 1000 ppm	53.4±4.6 ^b	18.5±3.6 ^b	21.5±2.9 ^b	64.0±8.8 ^b	66.1±6.7 ^b	32.5±11.4 ^b	68.5±7.7 ^b	91.3±3.34 ^c	108.6±4.4 ^c	129.1±4.9 ^c	139.4±5.5 ^c	156.8±6.2 ^c
SEm±	0.3	0.2	0.3	0.2	0.4	0.3	0.3	0.2	0.3	0.3	0.3	0.3
CD (at 5%)	0.8	0.6	0.9	0.6	1.0	0.8	0.7	0.6	0.8	0.8	0.7	0.7

VS, Vegetative stage; CIS, Curd initiation stage; FCS, Full curd stage; DAT, Days after transplanting.

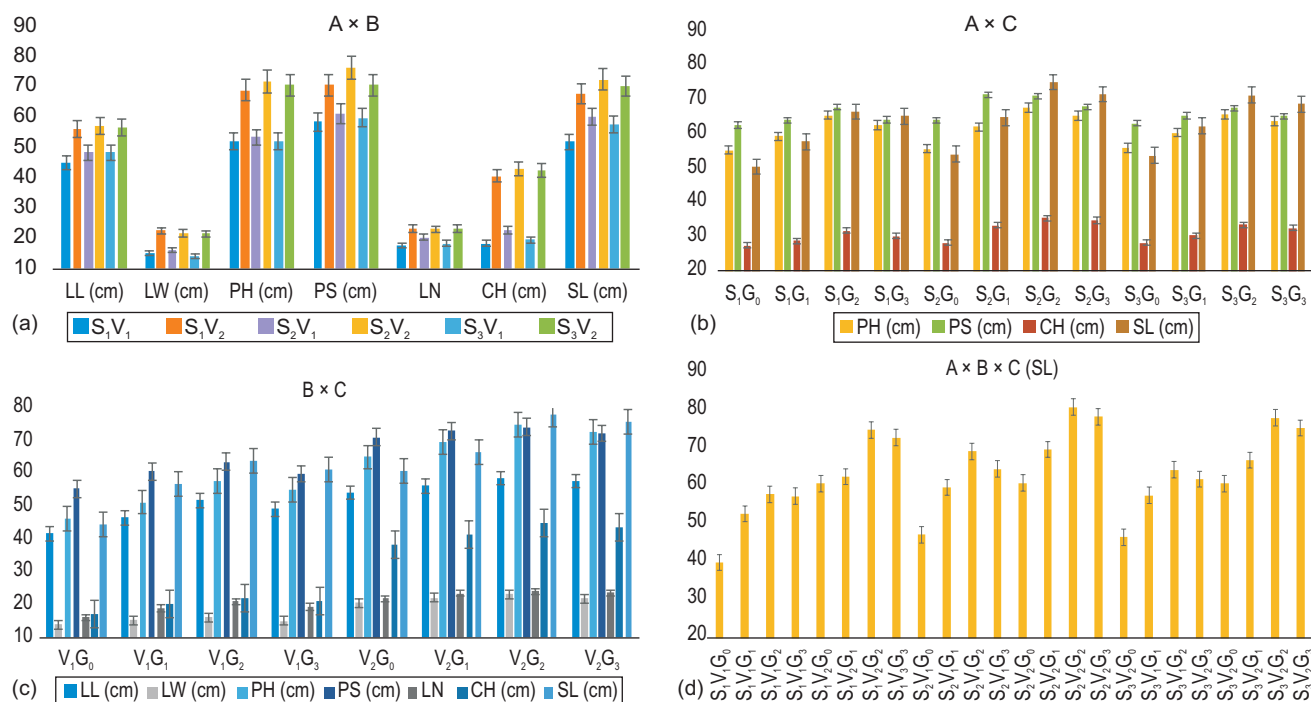


Fig. 1 (a–d) Interaction effect for morphological traits between stage of application and genotype (a); Stage of application and GA₃ concentration (b); Genotype and GA₃ concentration (c); and Genotype, stage of application and GA₃ concentration (d).

A, Stage of application; B, Genotypes; C, GA₃ concentration; LL, Leaf length; LW, Leaf width (cm); PH, Plant height; PS, Plant spread; LN, No. of leaves; CH, Curd height; SL, Stalk length (cm); S₁, Vegetative stage; S₂, Vegetative stage + Curd initiation; S₃, Vegetative stage + Curd initiation + Full curd stage; V₁, CF_{Or}; V₂, CF_{WT}; and G₀, Control; G₁, 250 ppm, G₂, 500 ppm and G₃, 1000 ppm.

(71.4 cm). The interaction between genotypes and GA₃ concentration was also significant for leaf length, plant height and spread, number of leaves/plant, curd height and stalk length (Fig. 1c). The highest leaf length (58.6 cm), plant height (74.8 cm), plant spread (74.2 cm), number of leaves/plant (24.1), curd height (44.9 cm) and stalk length (78.1 cm) was recorded in CF_{WT} at 500 ppm GA₃. The same level of GA₃ also resulted in the maximum values of the above stated morphological traits in CF_{Or}. These findings consistent with the findings of Prodhan *et al.* (2022). The interaction between application stage, genotypes and GA₃ concentration was significant only for stalk length (Fig. 1d and Supplementary Table 1). The longest stalk length was obtained in CF_{Or} (69.33 cm) with GA₃ (500 ppm) spray at VS + CIS stage. It was statistically similar to CF_{WT} (69.83 cm) with GA₃ (250 ppm) spray at VS + CIS stage.

Effect on developmental traits: All the developmental transitions were significantly influenced by GA₃ application stage except for days to curd initiation for pooled year data (Table 3). The minimum days to attain full curd stage (112 days) were observed when GA₃ spray was done at VS + CIS stage which was significantly similar to VS + CIS + FCS stage (113.1 days). Early bolting (132.6 days), flower bud formation (142.8 days) and 50% flowering (160.3 days) were also recorded with GA₃ spray stage at VS + CIS.

Between the genotypes, CF_{WT} took significantly lesser days for curd initiation (89.7 days), full curd stage (106.9 days), bolting (128.6 days), flower bud formation (138.5 days) and 50% flowering (156.0 days) compared to CF_{Or}.

Similar to morphological traits, the CF_{Or} also showed better response to days to curd initiation, full curd formation, bolting, flower bud formation and days for 50% flowering. In vernalization-responsive species, the GA activates *SOC1* gene when vernalization is insufficient to induce *SOC1* activation (Jung *et al.* 2020). The early onset of flowering in white cauliflower genotype could be linked to this phenomenon, however, it needs further investigation. *SOC1* integrates GA dependent flowering pathway which might explain the accelerated developmental transitions in CF_{WT}.

Among different GA₃ concentrations, early curd initiation (89.0 days), full curd formation (105.5 days), bolting (125.4 days), flower bud formation (135 days) and 50% flowering (151.9) was observed at 500 ppm. Around about 13 days, 17 days, 22 days, 24 days and 27 days difference was observed between the 500 ppm GA₃ treatment and control for curd initiation, full curd, bolting, flower bud formation and 50% flowering, respectively. GA enhances the transition from vegetative to reproductive stages in cauliflower. Early transition for curd development was agreed with Prodhan *et al.* (2022) and Nakajima *et al.* (2023). While, it contradicts the findings of Duclos and Bjorkman (2015) for other developmental transitions, possibly due to difference in the ecotype group of cauliflower.

The interaction between application stage and genotypes was significant for all the developmental transitions (Fig. 2a). Early full curd stage (106.5 days), bolting (126.8 days), flower bud formation (136.5 days) and 50% flowering (153.2 days) was observed in CF_{WT} with GA₃ spray at

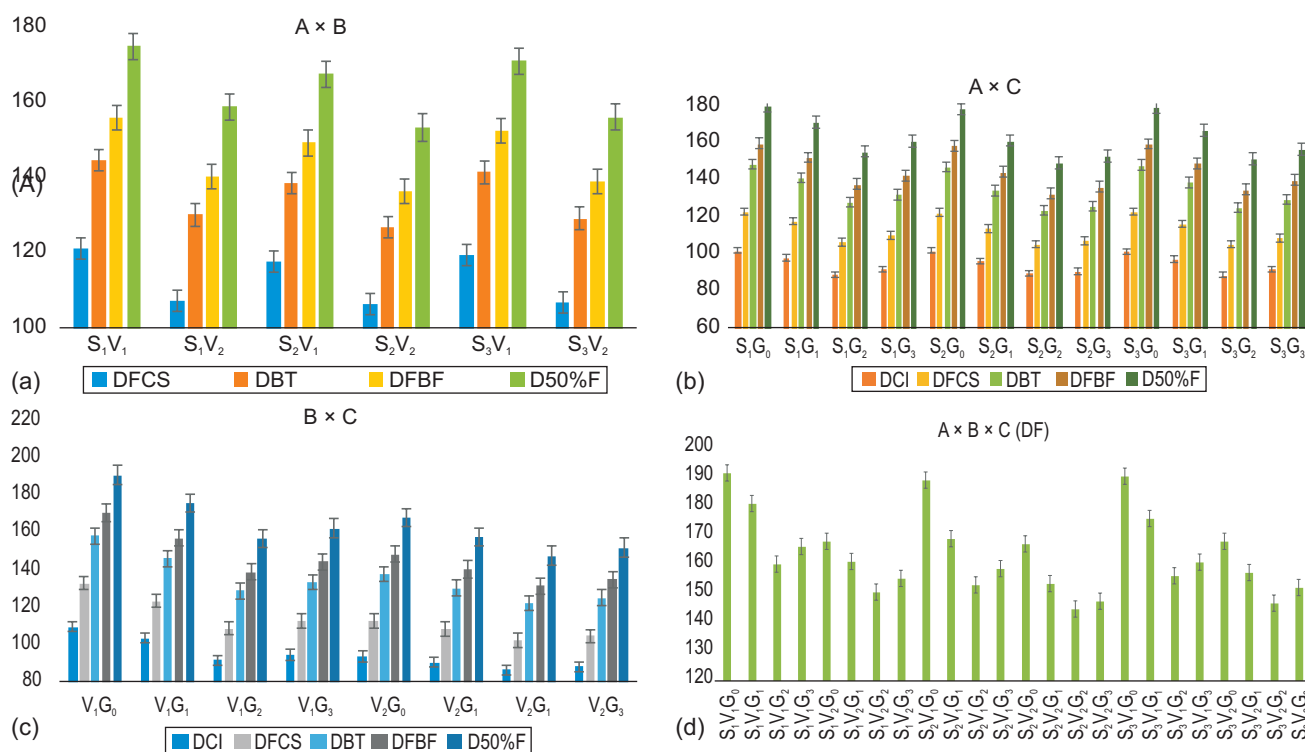


Fig. 2 (a-d) Interaction effect for developmental transitions between stage of application and genotype (a), Stage of application and GA₃ concentration (b), Genotype and GA₃ concentration (c) and Genotype, stage of application and GA₃ concentration (d). A, Stage of application; B, Genotypes; C, GA₃ concentration; S₁, DFCS, Days curtl stage; DBT, Days to bolting; DFBF, Days to flower bud formation; D50%F, Days to 50% flowering; Vegetative stage; S₂, Vegetative stage + Curd initiation; S₃, Vegetative stage + Curd initiation + Full curd stage; V₁, CF_{Or}; V₂, CF_{WT}; and G₀, Control; G₁, 250 ppm, G₂, 500 ppm and G₃, 1000 ppm.

VS+CIS stage. Similar trends were recorded in CF_{Or} at VS + CIS stage (117.5 days, 138.5 days, 149.1 days, 167.4 days, respectively), but there were significant differences between CF_{Or} and CF_{WT} irrespective of application stage. Guo *et al.* (2004) reported inflorescence stalk elongation in vernalized plants (10°C) treated with GA₃ (50 or 100 ppm) at 8–9 leaf stage, with less effect in non-vernalized plants (15 or 20°C).

Interaction between stage of application and GA₃ concentration has significant effect on all the developmental transitions (Fig. 2b). Days to curd initiation (88.6 days) were significantly less with GA₃ spray (500 ppm) at VS+CIS+FCS stage. While minimum days for full curd stage (105.0 days), bolting (123.4 days), flower bud formation (132.6 days) and 50% flowering (148.9 days) were observed from GA₃ spray (500 ppm) at VS+CIS stage.

The interaction between genotypes and GA₃ concentration was also found significant for all the developmental transitions (Fig. 2c). Minimum days taken for curd initiation, full curd stage, bolting, flower bud formation and flowering were observed in CF_{WT} with 500 ppm GA₃ which was significantly lesser than other treatments. The same treatment also resulted earliest attainment of developmental transitions in CF_{Or}. Earliness in curd maturity was also reported in mid group white curd cauliflower with spray of GA₃ (200 ppm) by Sonam *et al.* (2020) and in European type group with GA₃ @800 ppm by Yacoub and Abbas (2012).

Interestingly, the interaction between stage of application, genotypes and GA₃ concentration was significant for days to 50% flowering only (Fig. 2d, Supplementary Table 1). The flowering time of CF_{Or} with 500 ppm at VS + CIS stage (153 days) was coinciding with the flowering time of CF_{WT} with 250 ppm GA₃ spray at VS + CIS stage (153.5). Thus, two sprays of GA₃ were effective for desirable transitions instead of 16 sprays as reported by Hamano *et al.* (2002) in cabbage.

Overall, the study indicated that exogenous GA₃ treatment, particularly at the VS+CIS stage, accelerates vegetative growth and the transition to the reproductive phase, thereby shortening the growth period (Fig. 3). The study also suggested that the 500 ppm concentration is more effective in mid-late genotypes than the GA₃ concentration of 800 ppm in European group (Yacoub and Abbas 2012), 200 ppm in mid group (Sonam *et al.* 2020) and 250 ppm used by Prodhan *et al.* (2022). Further *Or* gene introgression might have also carried some genes of European material in recipient genotype, influencing the mid-late genotype's response to GA level. Consequently, the study prescribed a new level of spray for newly introgressed *Or* genotypes in mid-late group. The study concludes that effective dose of GA₃, particularly 500 ppm GA₃ applied at VS + CIS stage positively influences morphological traits and developmental transitions in *Or* gene introgression lines. It was found that the foliar application of 250 ppm and 500 ppm GA₃ at VS + CIS stage helps to attain the similar stalk length as well as synchronize the

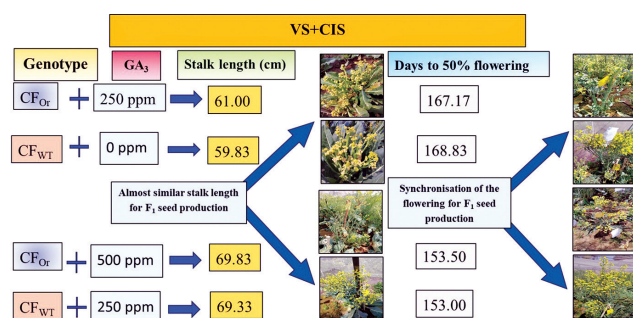


Fig. 3 Proposed model for synchronization of stalk length and days to flowering in white and *Or* gene introgressed Indian cauliflower.

VS, Vegetative stage; CIS, Curd initiation stage.

flowering time of CF_{Or} with the flowering time of CF_{WT} when sprayed with 0 ppm and 250 ppm GA₃ concentration, respectively. These findings contribute valuable insights for advancing the developmental transitions in the homozygous orange genotype of mid group which favour its use as one of the parents for hybrid seed production. However, the thermo-sensitive nature of cauliflower suggests for similar investigation to validate the findings in other groups (i.e. early and late maturity groups) of cauliflower for effective tapping of the *Or* gene in cauliflower biofortification.

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