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In vitro flowering in somatic nodal bud explants of the cultivated GI tagged Jasmine, (*Jasminum sambac* Linn.)

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Abstract
This report on *in vitro* flowering of the popular floral crop of *Jasminum sambac L.* presents interesting insights on a relatively unusual academically interesting spectacle where somatic nodal buds picked from field-grown shoots evinced flowering progressing up to anthesis. Reared on MS Basal Medium (BM) and BM supplemented with certain specific plant growth regulators (PGRs), nodal bud cultures initiated in the months of February and March elicited this response. With segments of floral receptacle and vegetative nodes excised from shoots priming for harvest refrained from displaying this phenomenon on hormone-free medium, while PGRs namely Gibberellic acid (GA_3) and Salicylic acid (SA) fortified to the medium helped the elicitation of this response. Results of the study show that certain endogenous stipulations and medium-specific conditions could find a role in triggering the response and influence variations in flower size and quality. Observation made in the different experiments of the study revealed that nodal buds isolated from the flowering twig had the propensity to differentiate a flower more in comparison with the juvenile flower buds induced *in situ* in floral receptacles. Concomitant development of both vegetative shoot and flower bud has been seen adjacently at opposing axils in a given nodal explant groomed medium with low level of SA implicating that flower production *per se* could be driven by an inherent stimulus facilitated with SA lest not exerting a determinative influence. Completion of flower development heading to anthesis and the lasting retention of floral organs for over four weeks defying withering is a feature of culture that prompts that the said PGR can be hired judiciously to mediate floral senescence.

Keywords: bud culture, shoot apices, nodal buds, floral induction, plant growth regulators, *jasminum sambac*

Introduction

Jasmine (Gundu Mallige, local name "*Madurai Malli*") is grown for its highly valued scent and elegance and it is the most important floral crop in India. Farmers in Madurai (India) earn their livelihood taking up jasmine cultivation as a full-time vocation. Planting Jasmine extensively in their fields and small holding, they engage in grooming, harvesting and trading this floral crop, irrespective of the monetary gain which comes from it during different seasons. The recently granted geographical indication (GI) tag helps them to sustain the market dynamics of the specific variety, even though their business flourishes for four to six month during the dry spell in a year. Flowering in Jasmine starts from the sixth month since planting but a reasonable economic yield can be expected only from the second year onwards. Unopened flowers at stages preceding anthesis are picked every day early in the morning and the market worth is curtailed for less than a day. A robust trading circuit helps the jasmine growers in the domestic market and there is ample scope for export of this fragile produce (Lavanya *et al.,* 2014).

Despite the hardships and risks, farmers in the region cultivate Jasmine quite intensively and continuously for the passion of preserving the socio-cultural sentiments attached to this ornamental. The perenniality of the crop is a solace to the investment as the hardiness of the species to endure drought allows the plant to tide over summer. Its versatility to monitor the environmental cues to provide for adjustments with the biotic and abiotic stresses can be attributed to the presence of a dynamic shoot apical meristem (SAM). SAM strikes a balance between continued vegetative growth and the ability to offer yield in the crop which in this case is the unopen flower bud. Studies on ornamentals such as Jasmine have not been taking up as much as research on other food and fruit crops (Gowdhami and Rajalakshmi, 2015; Mourya *et al.,* 2017).

In vitro flowering has been demonstrated in a quite good number of species (Murthy *et al.,* 2012) [31], although streamlined protocols have not been developed yet.. Mulin and Van (1989) [30] have demonstrated techniques to raise *Petunia* flowers *in vitro* from thin layer explants and the factors controlling the process have been examined in *Kalanchoe* by others (Yang *et al*., 1999) [53]. Successful attempts have been made in certain commercial crops (Hilson and La Motte, 1977; Nadgauda, 1997; Lin *et al.,* 2007) [12, 33, 44] ornamentals (Nell *et al.,* 1982; Sim, 2008) [35, 43] medicinal plants (Nitsch and Nitsch, 1967; Thiruvengadam and Jeyabalan, 2001; Jabeen *et al*., 2005) [36, 14] and food crops (Hisajima *et al*., 1987; Pierik *et al.,* 1994) [13, 37] that this unusual phenomenon is seen as a familiar event in culture. Salicylic acid (SA) has been used to stimulate *in vitro* flowering in aquatic plants such as *Wolffia* (Khurana and Maheshwari, 1983) [17], *Lemna* (Khurana and Maheshwari, 1978) [15], and

Spirodela (Khurana and Maheshwari, 1980) [16], and the practice of using this tannic substance is followed in organ culture studies. That SA is hired in the cut flower industry for prolonging the longevity of the produce (Hatamzadeh *et al* 2012) [11] and is used for inhibiting ethylene biosynthesis in pear cell suspensions (Leslie and Romani, 1988) [24] and tobacco cell cultures (Martin-Mex *et al.,* 2005) [27] prompted the use of this biostimulant in inducing flowering. Better recognized for the thermogenic properties (Yusuf, 2013) [54] and its role in disease resistance (Delaney *et al.,* 1994) [6], SA has been reported to trigger stress-induced flowering (Wada and Takeno, 2013; Thomas, 2006) [51, 47]. The recognition of the presence of SA in several of the agronomically important crops and the cognizance of its role in the florogenesis (Raskin, 1992) serves the basis for this study which reports *in vitro* in this important floral delight.

Materials and Methods

Plant material and source of explant

Two-year-old healthy, pathogen-free, actively growing plant stocks groomed well for flowering with regular agronomic inputs followed in a commercial gardens Parampatti at Madurai district, Tamil Nadu, India, served as the principal material for this study. In vitro cultures were established with compliance to the recommended aseptic procedure. Shoot apex, nodal and floral buds collected fresh from the field were used as explants in the experiments that were replicated adequately.About 40 explants in each category were brought to culture in a given treatment and the experiments were repeated not less than three times.

Surface sterilization

Every time before inoculation, the chosen explants were washed well in running tap water for about 5 min and again washed with distilled water thrice. Following this, the explants were surface sterilized by rinsing first with 70% ethanol and treatment using 0.1%-0.2% of mercury chloride solution for 3-5 min adhering strictly to the surface disinfection schedule in the laminar airflow chamber. Somatic explants shoot apices and nascent flower buds cut out from floral receptacles were introduced in cultures suiting the object of the study.

Culture conditions

Medium containing MS salts (Murashige and Skoog, 1962), supported with White's vitamins, 3% sucrose (wt/vol), and 100 ppm l-1 myo-inositol was used uniformly as the basic culture medium. Difco bacto agar was used as a gelling agent and was added at 0.8 to 1%. The pH was adjusted to 5.6-5.7 before the addition of the gelling agent. PGRs used in this investigation included: 2,4-Dichlorophenoxyacetic acid (2,4-D), Naphthalene acetic acid (NAA), 6-Benzylaminopurine (BAP), GA_3, Indole 3- Acetic Acid (IAA), and SA (concentration ranges from 0.1mM to 5mM) depending on the scope of the experiment. Reagent and fine chemicals were procured from standard companies such as Merck and Lobo chemicals and special attention was paid to conform to the standard laboratory procedure. Culture vials with media were sterilized in an autoclave at 15lbs for 15 min. Culture conditions were maintained at 16 hr light and 8 hr dark period with 3000 lux light intensity provided by cool white fluorescent tubes. The temperature of the culture room was kept at 25 ± 2° C.

In vitro treatment -1

As part of the preliminary investigation, trials were initiated on MS basal medium and a medium supplemented with (BAP 2µm and 2,4-D 1µm). Somatic explants, shoot apices from the flowering stalks and floral receptacles of varying maturity cut into 3 mm long segments and the un-open flower buds of three different stages, (i) pre-bloom stage (ii) nascent flower buds at balloon stage, and (iii) fluffy flowers closer to anthesis were used as explants. On a day to day monitoring, the first responses namely, change in pigmentation and increases or changes in the size and weight of the tissue were evaluated. Developmental changes such as callus differentiation, axillary bud development, and organized growth changes discerned from the exo-morphic projections are gauged regularly and were evaluated uniformly in all cases on the 28th day of incubation. Though representative sample tissues and flower buds from different cultures were dissected and microscopically examined to ascertain the state of maturation of constituent structures, color, and texture of callus and amenability of the callus and bud to proliferate upon subculture were considered as key traits in assessing the organogenetic capability.

In vitro treatment -2

Trials intended on *in vitro* flowering by considering variations in screening suitable explants and hormonal supplements were undertaken based on insights from the previous experiment. Three selected classical plant hormones and a PGR of a lesser-known kind Salicylic acid were tired and compared for their effects at selected concentrations and combinations. To narrow down fluctuations within the explant type, nodal bud collected were segregated as shoots excised from two different strata of the canopy namely bud reaching out to the surface directly exposed to sunlight and their counterparts confined to the lower strata where buds were under dissected shade were introduced in cultures. Shoots brought to culture from the surface of the field-grown shoots with direct exposure to sunlight (Class I) were differentiated from the counterparts (class II) picked from the side shoots emergent at the lower region that had access only to diffused light. With the former providing good results, bud and floral stalks picked from the surface were considered with special attention in the evaluatory trials.

Parameters of investigation

Growth and morphogenetic responses of the explants reared in cultures were tracked with special reference to the frequency of response in each treatment and growth and developmental changes showcased in individual flowers. Observable changes in the perianth whorl, changes in pigmentation, elongation, and expansion of the calyx whorl, time for anthesis, growth of the corolla tube besides changes in its pigmentation were considered. To ascertain deviations in flower quality field-collected samples at different stages of floral development were determined by determining changes and shifts in flower quality encountered in the *in vitro* simulations. Both lab and field-collected samples were serially sectioned in longitudinal planes and were examined to localize osmophores, the sub-ovarian cavities seated in the pedicel head.

Studies on salicylic acid

In a special bid to check the efficacy of SA was tested at additional concentrations with and without the accompaniment of GA3 and other PGRs at selected concentrations. The growth regulating metabolite is supplemented to the medium as in the case of other PGRs for as low concentration of 0.01µM to 2µM and its involvement in sustaining the leaf development, growth of axillary shoots and the flower production is evaluated. Though the effects on the keeping quality of the flower are not assessed in quantitative terms, the efficacy of the substance is qualitatively checked, especially with reference to the claimed role of SA as an anti-senescence agent.

Results

Initial observations made on source plants in the field in February showed that the nature and functions of the shoot apices in bushes differed in their size and responses differed based on microclimatic conditions where the given twig is positioned. Those buds reaching out for direct and intense exposure to sunlight elicited a higher propensity to be transformed into a floral meristem. In contrast buds and shoots placed at a lower level exposed barely either remained dormant or continued to form axillary shoots. Buds noticed at the axillary region of the nodes in juvenile and mature shoots also followed the same trend. Shoot apices and buds at the axillary region of the nodal explants by virtue of the maturity state of the leaves and subtending shoots differentiated flower buds and also formed secondary cymes.

Basic Responses

Observations made in the initial studies showed that the somatic and reproductive shoots from the Jasmine plant can elicit varied responses in cultures. While scant callusing and marginal enlargement of size in explants were evident in most explants in hormone-free basal medium, cultures reared on PGR supplemented medium evinced pronounced growth changes. Excised regions of the leaf tissues formed soft nodular wound callus at the petiole region while the leaf segment culture and leaf discs excised from the laminar region remained recalcitrant. In this primary experiment, nodal regions and floral receptacles sliced from the flowering axis showed an increase in size by stretching along the axis whereas in the flower buds cultured with their floral stalk showed the elongation pedicel and sepals in medium with PGRs. Conversely, shoot apices isolated from the juvenile and mature shoots could not acclimatize well in culture due to drying and desiccation. The nodal regions on the other hand responded well both on BM and PGR added medium. Among the different explants, nodal buds differed in eliciting two different types of responses. As the growth of lateral shoots in the axillary regions in selected cases, a leafless stalk subtending a sub-hemispherical structure resembling flower buds could be noticed at low frequencies. Upon repeated trials and closer examination it was found that nodal regions appearing to be primed in nature to be initiated as floral receptacles could be fostered to continue development differentiating flower-like protuberances with perianth whorls(Table1). This response is unique in medium fortified with BAP and 2, 4-D led to a next level investigation involving PGRs.

Table 1: Basic responses on explants of Jasminum sambac L.

Explant Type	Growth responses in MS medium with and without PGR augmentation									
	Change in colour		Expansion/ elongation		Callus Development		Axillary Bud Growth		Floral Bud Initiation	
	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
Shoot apex	Green	Brown	0	0	-	-	0	+	-	-
Segmented leaf	Green	Yellow-green	0.2	1.6	+	++	NA	NA	-	-
Leaf Disc	Pale brown	Pale brown	0	0	-	+	NA	NA	-	-
Nodal bud	Green	Green	0.5	1.8*	+	++*	1	2	-	+
Floral receptacle	Green	Green	0	1	+	+++*	0	0	-	-
Floral bud 1	Green	Green	0.5	1.6	+	-	0	0	-	-
Floral bud 2	Green	Green	0.5	2.3	+	++	0	0	-	-
Floral bud 3	Green	Yellow-green	0.5	3.8	+	+++	0	0	-	-

Medium: (A) MS Basal Medium; (B) MSBM + BAP2µm and 2, 4-D 1µm.

*Basal region proximal to the excised surface is considered; Colour Change as visually observed: Expansion/ elongation-increase alone shown in mm, for flower bud increase in sepal length is also included: Callus development assessed in terms of quantum increase + means upto 20% increase in original size; ++ 20-50 increase; +++ more than two fold increase in original size: Axillary Bud Growth -NA not applicable; numbers as counted per explant: Floral Bud Initiation assessed as a present (+) and absent (–).

Hormone mediated changes

Since callus development and lateral bud differentiation prevailed as dominant responses despite wider fluctuations, further experiments focusing on segregating the explants based on shoots from where they were emerging in the source plant and investigations ascertaining the effects of PGRs as supplements were carried out in phase II experimentation. Figure 1 presents an overview of the responses of different explants brought to the culture. While medium fortified with GA_3 unfolded in its first pair of leaves (Fig.1.a) nodal segments at comparable conditions managed to its active bud differentiation at the axils (Fig. 1b). Comparisons made between the flower buds and floral receptacle (the stalk that ought to bear the flower), and the nodal bud showed contrasting changes concerning the PGR additions. Young floral receptacles excised and reared in culture on medium containing 2um GA_3 developed flowers from the pre-differentiated buds at the distal ends (Fig.1.c), mature somatic nodes (3rd for the shoot tip) elicited accessory bud differentiation (Fig.1.d) and formed callus at the excised basal ends when additionally fed with 2, 4-D in the medium (Fig.1.e).

Comparison of responses between nodal regions of somatic shoots and flowering axis showed that 2 µm NAA

containing medium can induce callus at the proximal ends in both types of explants as the calyx whorl showed a marked increase in auxin supplemented medium, individual sepals widened laterally to form a sheath or a rosette (not shown in the figure) in GA_3 fortified medium. While corolla whorl could find space for its development in most treatments where 2,4-D is used as an additive, almost floral whorls including corolla, androecium, and the gynoecium callused irrespective of the influence of the co supplement. As a higher concentration of auxins, especially 2,4-D (2 µM) suppressed the formation of a flower, in medium with lower doses (0.2 µM) the sepals in the cultured flower bud could give rise to soft off-white to yellow colored calli.

Leaves and the nodal buds callused in response the same way to the said PGRs. As the axillary bud differentiation and lateral shoot formation in nodal explants prevailed in nodal explants in BAP and GA_3 augmented media, especially in the absence of 2, 4-D, floral receptacles remained largely recalcitrant. Flower buds explanted at too early stages showed the formation calyx but the epipetalous corolla tube and the gynoecium failed to emerge in a comparable medium. Even though the cytokinin BAP and GA_3 could engage in favoring organized growth in the pre-existing primordia in nodal buds and floral receptacles (Table2), only GA_3 and SA combine enabled *in vitro* flowering in somatic explants. The *in situ* differentiated flowers brought to culture and the floral receptacles at the verge of flowering that showed no developmental deviation in response to the hormonal supplements provided in the medium. Trials pursued with the culture of somatic nodal buds provided clues to speculate that precocious flowering could take place if SA and GA are used as growth supplements (Table 3).

Table 2: Plant Growth Regulator induced growth changes in nodal explants (A) and floral receptacle (B) of Jasminum sambac L.

PGR supplementation	***Callus Development***								***Shoot proliferation***					
	Colour		**Texture**		**Magntitude**		**Prolif.**		**Ax.bud**		**Ax. shoot**		**Fower bud**	
	A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
BM	-	w	-	N	-	+	-	-	+	-	-	-	-	-
BM + 2µM NAA	Y	Y	C	C	+*	+	s	L	-	-	-	-	-	-
BM + 2µM BAP	-	-	-	-	-	-	-	s	+	-	+	-	-	+
BM + 5 µM BAP + 0.5 mM 2,4-D	G	Y	C	S	+	+	A	A	-	-	+	-	-	-
BM + 2µM 2,4-D	Y	w	C	S	++	++	A	P	-	-	-	-	-	-
BM + 0.2 µM GA_3	-	-	-	-	-	-	-	-	+	-	-	-	-	+
BM + 2 µM GA_3	-	-	-	-	-	-	-	-	+	-	+	-	-	+
BM + 1µM GA_3 + 0.2 µM 2,4-D	Y	Y	C	S	+	++	s	A	+	-	+	-	-	+
BM + 1 µM GA_3 +1µM BAP +1µM 2,4-D	G	Y	C	C	++	+++	A	P	-	-	-	-	-	+
BM + 2 µM GA_3 + 1 µM BAP+ 1 µM NAA	Y	G	H	C	+*	+	s	A	+	-	+	-	+	+
BM + 1 µM SA + 0.2µM GA_3	-	-	-	-	-	-	-	-	+	+	+	+	+	+
BM + 1 µM SA + 0.2 µM 2,4-D	G	Y	C	C	+	++	A	P	+	+	-	-	+	+

Explant: A nodal explant, and B floral receptacle. Colour change: visually observed and matched with standard colour gird: w-off white, Y-yellow, G green; Texture: N-nodular, S-soft, C- compact, H-hard; Magnitude: callus assessed in terms of quantum increase (- Nil; '+' upto 20% increase from the original size of explant; '+*'callus confined to the basal cut end; '++' 20- 80% increase; '+++' more than two-fold increase in size): Prolif. –Proliferative ability as noted in the 3rd week of incubation s- Scant, L-Low, A- Active, P-Profuse: Shoot proliferation assessed in terms of the emergence of bud (somatic- Ax.bud & flower bud), and and absence of leafy shoot (Ax. Shoots) '+' indicates presence & '– 'absence)

Table 3: Influence of Salicylic acid on in vitro flowering Jasminecultures.

Medium	**Responses in nodal buds in a single node**							
	Leafy shoot formation		**Floral Transistion**		**Flower Development**		**Co induction of shoot and floral bud**	
	I	**II**	**I**	**II**	**I**	**II**	**I**	**II**
BM	1	1	-	-	-	-	-	-
BM + 0.1 µM SA	1	1	+	-	*If*	*if*	+	-
BM +1 µM SA	1	1	+	-	*Mb*	-	+	-
BM + 10 µM SA	2	2	Partial*	-	*af*	-	-	-
BM+ 1µM SA +0.5µM BAP	3	3	-	-	-	-	-	+
BM + 0.1 µM SA +2µM BAP	0	0	-	-	*if*	-	-	-
BM + 1 µM SA +0.5µM GA_3	0	1	+	+	*Mb*	*mb*	-	-
BM + 1 µM SA +0.5 µM 2,4-D	0	0	-	-	*Ab*	-	-	-
BM + 0.5 µM SA +2 µM GA_3	1	2	+	+	*If*	-	+	-

BM + 2 µM SA + 2µM GA_3	2	2	+	+	-	-	-	-

Explant Class: I Orthotropic shoot at the surface of the canopy; II Plagiotropic side shoots emerging from older regions closer to the ground. In all cases, it is the third nodes counted for the tip that is used as explant. Leafy shoot formation assessed in terms of a number of leaf pair formed: Floral Transition –noted for the presence semi flowers or brownish bract-like leaf formation with a whorled character of a flower (+ presence and – absence); Flower Development assessed based on the nature of a floral bud (*af* -abnormal bud; *if* incomplete flower bud; *mb* mature bud) Co-induction of the shoot and floral bud assessed as + present or – absent.

Fig 1: Glimpses of PGR induced changes in cultures of Jasminum sambac L. Growth changes showcased in shoot tip (a), nodal bud (b) and floral receptacle (c) revealing the accessory bud differentiation in medium with BAP (d), callus development and 2.4-D (e) being contrasted with GA induced shoot development (f). Flower development in buds of flower bud stage I (g) fostered on BM compared with a bud (stageII) and (stage III) groomed in GA3 containing medium without (h) and with the accompaniment of 2,4-D (i) respectively paced in contrast to the organized florogenesis in SA treatments (j-p).

Salicylic acid influenced changes

Buds in the axillary region of the single node cultures in SA medium presented the feasibility of developing active axillary buds, leafy lateral shoots, and structures that exomorphically resembling flower buds at variable frequencies (Fig. 1.j). *In vitro* cultures initiated fresh in February and March presented such a mixed bag of response. Among the various types of growth supplements, GA_3 and SA were introduced as additives fetched certain unique developmental changes. That flowering in somatic explants was witnessed the only medium fed with SA helped to focus on narrowing down deviations in the second phase of experiments. Besides focussing on fine-tuning the PGR supplements, efforts were directed in segregating the explants class based on (i) the maturity state of twigs collected from the field to be used as a source of explants and the (ii) nodal number from within the shoot from which the explants were prepared.

It was interesting to note that that shoots picked from the surface of the canopy could offer much better results than source twigs collected at the proximal regions. It was evident from the observations of the study that on a comparative basis nodal bud explants picked form the former class responded in a larger number with better results. Microdissection of floral parts made form samples in selected treatments showed that at least three different types of flowers could be formed *in vitro*. With the lowest concentration and higher level supplementation of SA drawing a blank, the substance at 1µm concentration with and without the co-supplementation of GA_3 formed regular

flowers with all four floral whorls. Contrasted form this is the case of flowers that formed calyx and pistil lacking the epipetalous second whorl which is described in the present context as incomplete flowers.

Deviant from this is yet another type of flower that lacked part of calyx but had crushed corolla and an inflated gynoecium (Fig.1.l). As against this response noticed in medium with 0.1µM SA and 2µM BAP, culture reared with 1µM SA and 0.5µM 2,4-D distorted the differentiation of the regular floral whorls. Referred in the study as abnormal flowers these emergent structures exhibited an unusual enlargement of the ovarian and sub-ovarian tissues. The longitudinal section made in samples revealed that two cavities are presumed to be osmophore is located in this region. Though uncommon another observation of interest could also be made concerning the third node counted from the terminal cyme. Two buds adjacently placed in opposing axils of a given node could reel out divergent responses. As one of the buds mentioned here had formed a flower its counterpart remained somatically active forming a foliar shoot with broad unfolded leaf. Flower buds of stage III groomed in medium fortified with 1 µM SA and 0.5µM GA_3 could elicit noticeable elongation of the gamopetalous corolla tube. Although prominent anther sacs were spotted in such flowers, *in vitro* raised flowers invariably lacked functional pollen grains.

Table 4 is a summary of observations made concerning changes inflicted on perianth and essential organs of the flower. Despite the higher percentage of successful establishment, stage I flower buds remained largely recalcitrant. In contrast, stage III flower bud displayed the inherent growth changes and elicited anthesis *in vitro*. In stark contrast floral longevity observed in nature where the corolla tube senescences and withers away in 24 hours from the opening of the flower, floral whorls formed in SA treatments remained intact for several weeks. Random analysis of the samples drawn from *in vitro* cultured flower buds showed that both the anther and the ovary were invariably empty. Differences in the size of these organs could be related to PGRs inputs provided in the medium, although such changes my not hold any significance in Jasmine as the flower in itself turns out to be the produce.

Table 4: PGR influenced changes in perianth whorl and anthesis in stage II flower buds.

Medium	**Change in the perianth whorl**					**Anthesis**	**Changes in Essential organs**	
	Calyx		**Corolla**				**Increase in size Anthers**	**Increase in size of ovary in Pistil**
	Colour change	**Elongation**	**Increase in width**	**Pigmentation after 3 weeks**	**Corolla elongation*/enlargement**			
BM	Green	+	-	Brown	-	-	+	+
BM +2µM NAA	Pale Green	+	-	Grey	-	-	-	-
BM + 2µM BAP	Dark Green	++	-	Yellow with green tinge	-	-	-	-
BM +2 µM BAP + 0.5 µM 2,4-D	Yellow green	+(c)	-	Purple	-	-	-	+
BM + 2µM 2,4-D	Green	+(c)	-	Brown to Purple	-	-	-	-
BM + 2µM GA_3 + 0.5µM SA	Green	+	+	Off-white	+*	-	+	-
BM + 2 µM GA_3	Green	-	+	Off- white	+*	-	+	-
BM + 0.01 µM SA	Green	-	-	Off white	-	-	+	-
BM +0.05 µM SA	Green	-	+	Off white	_	+	+	-
BM + 0.1 µM SA	Green	-	+	Off white	+	+	+	-
BM+ 1µM SA	Green	-	+	Off white	++	+	+	-
BM +2µM SA	Green	-	++	Off white	+	+	-	-
BM + 2µM SA + 1µM BAP	Green	-	+	Yellow green	++	+	-	-
BM +2µM SA + 1µM GA_3	Green	-	++	Grey	+*	+	-	-
BM +2µMSA + 1µM NAA	Green	+	-	Yellow	-	-	-	-
BM +2µM SA + 1µM 2,4-D	Yellow	+(c)	-	Purple	-	-	-	++

Perianth *Calyx*- Colour change in sepal as visually observed: Elongation – no increase in basic size from 10 mm assessed in terms of quantum increase (+ means 1-3mm than original size; ++ more than 3mm increase); Enragement – widening of sepal (- Nil; + increase by 1-2mm; ++ more than 2mm: *Corolla* - Colour change in freed part of petal as visually observed; Enragement – widening of petal fused region ignored, free part is 12 mm broad; + up by 3 mm; ++ 3mm Essential whorls: Increase in size of Anthers (- indicate absence & + Present): Enragement of Ovary (-no increase, + marginal increase + more tow fold increase in width.

Discussion

A flower being considered in classical terms as a condensed and modified shoot mandated as a composite and culminating product of somatic development is indeed a fine-tuned preposition for the act of reproduction aimed at ensuring recombination and a genetic mix catering to the survival strategy of land plants. Ensuring internalization of fertilization and negating elaborate gametangial constructs, floral evocation is timed with the conversion of apical meristem to mantle and provides for synchronous and simultaneous development of floral whorls. The circuitry of plant development which operates through SAM functions is complex in sympodial perennials unlike in monopodials, annuals, and moncarpic species. Unlike in latter taxa where florogenesis is considered a terminal event setting the stage for aging and senescence, floral evocation recurs in dicotyledon perennials year after year on maturity in the season to which the plant is accustomed. While the structural alterations which follow the conversion of SAM to floral meristem is irreversible, perennials control their decentralized flowering behavior through a complicated switch and clock mechanism (McClung, 2001).

It can be inferred from this study that in Jasmine, SAM is more versatile and fragile. It is but a common understanding that floral induction is a chiseled and sensitive process where SAM responds with specific references to the environmental signals, though it might not directly receive the stimulus as shown in studies on photoperiodism (Ma,1998) and thermoregulation (Kovac and Stabentheiner, 2011). Signals perceived by the leaves and appendiculate

structures are transmitted and transduced suitably that the SAM function is duly modified for flowering (Trewavas and Malhó, 1997). Although this generalization holds good for many, Jasmine meristem does not appear to follow this *sensu stricto*, as stray flowering is noticed all-round the year.

Efficacy of SAM in Jasmine

This study seeking to report *in vitro* flowering in the popular commercial variety, *J. sambac* L. (*Madurai Malli*) show that certain PGRs have the potential to evoke flower formation in floral receptacles and somatic nodes as well. Although vegetative organs have shown callus development and shoot proliferation to auxin and cytokinin as expected of them (Neibaur *et al*., 2008) [34], gibberellins and salicylic acid proved their grit in mediating the flowering response (King *et al*., 1987: Cleland *et al*., 1982) [18]. As shown earlier, 2, 4-D is seen to be more potent in inducing callus formation than NAA (Krans *et al*., 1982) [20]. With the callus triggered by surgical incision, wound calli proliferated well at culture-specific conditions where incorporation of optimal concentrations of PGRs has triggered callusing across the differentiation state and the physiological maturity of explants.

Shoot emergence known to be an exclusive event of the somatic nodal explants in many plants (Sivanesan and Jeong, 2007) [45] appears to be modified towards flower formation in Jasmine at selected conditions. Though the preformed primordium or region potentially designated to form axillary buds are prone to form lateral shoots, direct floral induction could be recorded in the floral receptacles in SA treatments. Since no other region elicited bud differentiation and organogenesis, Jasmine appears recalcitrant to adventitious shoot and root development. It is surprising that Jasmine which is propagated through vegetative cuttings and rootstocks compensating the lack of effective sexual propagation in field conditions, stayed short of being amenable to alternate methods of organogenesis. Matching expectation, increasing concentrations of BAP showed a proportionate increase in bud differentiation (Table-A.1). Leafy shoot development and repetitive bud differentiation favored by the cytokinin prompts the reconciling influence of the PGR in neutralizing apical dominance enforced by auxins (Lakshmanan *et al*., 1997) [22]. Conversely, GA_3 conferred with the role in promoting seed germination (Urbanova and Leubner-Metzger, 2018) [49], stem elongation, especially the intermodal extension (Zeevaart, 1993) [56], flowering (West and Phinney, 1959) [52], leaf and fruit senescence (Fletcher, 1969) [9] is ascribed with more direct influence in floral stimulation. Offering provisions to substitute photoperiodic stimulus (Mutasa-Göttgens and Hedden, 2009) [32], the hormone is said to enhance stress tolerance (Vettakkorumakankav *et al*., 1999) [50]. Daoust *et al.,* (1995) [4] observed that GA_3 is known to evoke flower production in cultures. Results of the present study is in direct alignment with this as flowering has been successfully induced in cultures of somatic nodes and floral receptacles. As the sensitivity of Jasmine to respond to the cytokinin BAP can be corroborated to earlier known ideas of cytokinin favorably influencing cell division, cell differentiation along with auxins, the deference of senescence and its ability to counter apical dominance. Nevertheless its role in *in vitro* flowering appears limited. The result presented in Table-A.2 & A.3 also reflects this.

Salicylic acid can step up in vitro flowering

It is found that SAM in Jasmine can time, control, and regulate flower development following the developmental design conferred upon it. While it is observed that florogenesis remains unperturbed even on BM due to the carry over the influence of factors prevailing in floral receptacles, the prowess to induce flowering in a vegetative node is clearly conferred by Salicylic acid applications. SA stood out from other PGRs in this study producing well-formed shoots and flowers (Table-A.3). Though not in Jasmine, similar observations have been made earlier by Ram and Mehta (1978) [39]. Best results of leaf expansion and ability from leafy shoots with buds at active state growth can be demonstrated in medium supplemented using SA, with and without the accompaniment of BAP. Data gathered in the present context augers well with the notion that SA in conjunction with BAP and GA, and also in solitary supplementation, can support both shoot growth and flower induction (Cleland and Ajami, 1974; Raskin, 1992) [2, 40]. Repeated trials of this investigation showed that the stage in which the culture is done makes the difference in determining the role of SA in Jasmine cultures. When added as a component in the medium, SA induced and sustained growth of pre-existing shoot buds and in addition formed several accessory shoot buds. In a medium with minimal concentrations (0.1µM), SA could support the formation of prominent shoots with well-spaced nodes and leaves on each axis. Such shoots could be dissected out and tried for rooting. Salicylates have been reported as an important adjuvant in culture studies performed on Coffee, Bean, *Ziziphus spina-christi*, (Quiroz-Figueroa, 2001; Zaghlool, *et al,* 2001; Galal, 2012) [38, 10]. It can be reckoned from the observations of this study that the favorable impact the SA created in shoot development can be regarded to be even better than that of BAP and GA3.

Lower concentrations of SA helped concomitant development of both shoots and flowers from buds that were positioned adjacently even within a single node suggesting that SA could be involved in priming buds inherently poised to elicit a response. Higher doses of SA added alone or in combination with GA_3, evinced a convincing influence on the quantum and quality of the flowers formed (Table-A.4). It could be gathered from the data presented that the effects of SA are concentration-dependent and the response is seen conspicuous on perianth than the essential parts of the flower. Viewed from the non-breeders point of view, the absence of pollen and the prominent ovules within ovary on *in vitro* raised flowers may not be of significance, as the application of SA and GA could suffice the expectation in favoring the formation of larger flowers. Floral whorls formed in the SA medium remained intact and fresh for a prolonged period of time.

The notable changes in the sepal length and the pedicle head (thalamus region) is seen enlarged in certain treatments *shows that the* supplemented PGRs have influenced changes in the size. However, it remains to be seen if the aroma component spinning around linalool production can be enhanced. It is reported that linalool was released from the osmophore seated to the tubular base of the corolla as two distinct points (Elumalai *et al.,* 2010) [8]. With fragrance reported to emanate from the base of petals in the harvested produce, the reported feature of hypertrophic swelling observed in culture may demand further investigations. Normally scent production coincides with anthesis and lasts

only for hours as the drying and senescence take a toll on the lasting odor of Jasmine fragrance. That it is found that the crumbling of petals and senescence is deferred in SA treatment and that the flower size is incremented in select treatments can be a trait of market value if clues of value added programs can be inferred from the studies with plant growth hormones.

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