Gasexchangeparametersandchlorophyllcontentasinfluencedby different chemicals and planting materials in pomegranate (*Punicagranatum*L.)cv.Bhagwa

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ABSTRACT

Soil drenching with paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment to tissue cultureplantsimprovedgasexchangeparametersviz.,photosynthetic rate(P)atbothflowering(17.15µmolm^{-2} s^{-1})andfruitsetstages(11.62µmol$m^{-2}s^{-1}$),transpirationrate(E)atbothflowering(7.16mmol$m^{-2}s^{-1}$)andfruitset stages(4.27mmol$m^{-2}s^{-1}$),stomatalconductance(g)atbothflowering(0.53mol$m^{-2}s^{-1}$)andfruitsetstages(0.28 mol$m^{-2}s^{-1}$).Hightotalchlorophyllcontentwasobservedduetosoildrenchingofpaclobutrazol@0.375ga.i.m^{-1}canopydiameter60daysafterbahartreatmenttotissueculturepla ntsatbothflowering(3.03mgg^{-1})andfruitset (2.00mgg^{-1})stages.Foliarsprayofnitrobenzene@2.0mllitre^{-1}totissueculturepla ntsimprovedgasexchange parameters viz., photosynthetic rate (P_N) at both flowering(17.52 µmol m^{-2} s^{-1}) and fruit set (14.05 µmol m^{-2} s^{-1}) stages.Whiletranspirationrate(E)washighintissueculturepla ntsduetosoildrenchingofpaclobutrazol@ 0.375ga.i.m^{-1}canopydiameter45daysafterbahartreatmentatfloweringstage(8.01mmolm$^{-2}s^{-1}$)whileatfruit set stage, soil drenching of paclobutrazol @ 0.375 g a.i. m^{-1} canopy diameter 60 days after bahar treatment to tissue culture plants registered highest transpiration rate (E) (4.07 mmol m^{-2} s^{-1}). Highest stomatal conductance (g) (g_s46 mol m^{-2} s^{-1}) was observed due to foliar spray of nitrobenzene @ 2.0 ml litre^{-1} to tissue culture plants at flowering stage while at fruit set stage, the same chemical registered higher value (0.26 mol m^{-2} s^{-1}) in grafted plants.Hightotalchlorophyllcontentwasobservedduetofoliarsprayofnitrobenzene@2.0mllitre^{-1}to tissue culture plants at both flowering (3.07 mg g^{-1}) and fruit set stages (2.94 mg g^{-1}).

***Keywords*:**Gasexchangeparameters,nitrobenzene,paclobutrazol,photosynthesis,pomegranate

INTRODUCTION

Sunlight is the primary source of energy for photosynthesis, although air temperature and humidity have an impact on transpiration. Transpirationiscausedbythedryingpowerofthe atmosphere, which depends on wind speed and relative humidity, as well as the evaporative demand fromnetradiationabsorbedbyleaves(Elanchezian *et al*., 2015).By allowing CO_2 and water vapour to pass into and out of the leaf, stomata play a significant role in controlling transpiration and photosynthesis(Mokhles*etal*.,2019).According toMonerri*etal*.(2011),stomatalaperturecontrol isinfluencedbystatevariables(suchasleafwater potentialand intercellularcarbondioxide concentration),the interactionof processes (transpiration and photosynthetic rates), and environmentalfactors(specifically,thedifference in water vapour concentration between the leaf surface and the bulk air).

Theamountofchlorophyllintheplant,together withthephotosyntheticrate(P_N),transpiration(E), stomatalconductance(g_s),andotherfactors,all affectthemetabolicactivityoftheplantandits overallgrowthanddevelopment.Therehavebeen extensivestudiesconductedonavarietyoffruit

crops, including mango, citrus, apple, sweet cherry, etc. (Jones, 1992). In an experiment with olive, Arun *et al*. (2017) looked at how plant growth regulators affected gas exchange and chlorophyll content.

Forthefirsttimeinpomegranate,studiesongas exchange parameters—photosynthetic rate (P_N), transpiration(E),andstomatalconductance(g_s)—aswellaschlorophyllcontentwerecarriedoutto examine the metabolic activity of the plant using chemicalslikemethyljasmonate,nitrobenzene,and paclobutrazol at different concentrations.

MATERIALSANDMETHODS

Thecurrentstudywascarriedoutattheresearch farm of ICAR-Indian Institute of Horticultural Research(IIHR)inHesaraghatta,Bengaluruduring the *ambe bahar* (January–February) and *hastha bahar* (September–October) seasons of 2020 and 2021.Asnotedbefore,plantsweremultipliedfrom threedifferentsources:airlayers(P_3),graftedplants (P_2), and tissue culture plants (P_1). During *Ambe bahar*, the average maximum and minimum temperaturesof 33.08^{0}C and 20.43^{0}C, were recordedrespectively,andtherelativehumiditywas 75.04 percent and 59.06 percent and the total rainfallwas74.95mmof12.10mm,respectively. During *Hastha bahar*, the average maximum and minimum temperatures of 26.13^{0}C and 18.94^{0}C were recorded respectively and relative humidity of 59.06 percent and the total rainfall was 12.10 mm,respectively.Aten-treefactorialrandomized blockstatisticaldesignwasemployed.Thestudy included three distinct concentrations of MeJA (MethylJasmonate),NB(Nitrobenzene),andone concentrationofPBZ(Paclobutrazol)delivered 30 days,45days,or60daysafterthe‘bahartreatment’. Thetreatmentswereexpressedasfollowsaccording totheconcentrationofacertaingrowthregulator: T_1: 100 ppm MeJA, T_2: 150 ppm MeJA, T_3: 200 ppmMeJA,T_4:1.0mlNB,T_5:1.5mlNB,T_6:2.0 ml NB, T_7: Soil drenching of paclobutrazol @ 0.375ga.i.m^{-1}canopydiameter30daysafterbahar treatment, T_8: Soil drenching of paclobutrazol @ 0.375ga.i.m^{-1}canopydiameter45daysafterbahar treatment, T_9: Soil drenching of paclobutrazol @ 0.375ga.i.m^{-1}canopydiameter60daysafterbahar treatment, T_{10}: Water spray.

Gasexchangeparameters

ThePortablePhotosynthesisSystem(LCpro+, ADCBioScientificLimited,UK)wasusedtoassess the ratesof photosyntheticactivity (P_N), transpiration (E), and stomatal conductance (g_s). Fullydevelopedleaveswereremeasuredatleastthree timesbetween9:30and11:30amwhiletheywere exposed to ambient light and CO_2 levels.

Totalchlorophyllcontent

Withtheaidofacetoneanddimethylsulphoxide (DMSO), the complete chlorophyll content was removed.A0.1gleafsamplewasdissolvedin10 ml of DMSO:Acetone reagent (1:1) and let to sit for 72 hoursin the dark. Usinga UV-VIS spectrophotometer (T80+ UV/VIS spectrometer, PGInstrumentLtd.,UK),theextractwascollected and the absorbance measured at 663 nm and 645 nmforquantificationofchlorophylla,chlorophyll b, and total chlorophyll. Additionally, using the equationsputoutbyLichtenthalerandBuschmann (2001), the pigment contents were computed and given in mg g^{-1}.

$$\text{Chlorophyll } a = [12.7(A_{663}) - 2.69(A_{645})] \frac{V}{1000 \times W \times a}$$

$$\text{Chlorophyll } b = [22.9(A_{645}) - 4.68(A_{663})] \frac{V}{1000 \times W \times a}$$

$$\text{Totalchlorophyll} = [20.2(A_{645}) + 8.02(A_{663})] \frac{V}{1000 \times W \times a}$$

Where,

A=Absorbanceatspecificwavelengths (645 nm and 663 nm)

V=Volumeoftheextract(10 ml)

W=Freshweightofthesample(100mg) a = path length of light in cuvette (1 cm)

Statisticalanalysis

The method of evaluating variance developed byPanseandSukhatme(2005)wasusedtoanalyze thedata.TheFvalueata5%levelofsignificance wasusedtodeterminethestatisticalsignificance. Criticaldifferenceswerecalculatedatthe0.05level to determine if there were any significant effects.

RESULTSANDDISCUSSION

Rateofphotosynthesis

Therateofphotosynthesis(P_N)intissue-culture plantswasconsiderablyimpactedbytheflowering stage of *ambe bahar* and reached its peak (14.24 $molm^{-2}$ s^{-1})inP_1(i.e.,intissue-cultureplants)(Table 1).Paclobutrazol,oneofthechemicals,produced a$P_N$of14.71molms60daysafterthebahar treatment(T_9),whichwascomparabletothose obtainedfromthesametreatment45daysafterthe bahar treatment (T_8) (13.89 mol m^{-2} s^{-1}) and nitrobenzene1.5mllitre^{-1}(T)or2.0mllitre^{-1}(T). P_1withpaclobutrazol45days(T_8)or60days(T_9) afterthebahartreatmentregisteredcomparable valuesofP_N,whereashighervaluesofP_N(15.46 mol ms^{-2-1}) were recorded in grafted plants (P_2) sprayedwithnitrobenzene2.0mllitre^{-1}(T).These differencesin$P_N$werealsosignificantbetweenthe types of propagules and chemical combinations.

Incomparisontotissue-cultureplantsorgrafts, air layers in ambe bahar displayed significantly higher$P_N$valuesatthefruitsetstage.Paclobutrazol amongthechemicalsshowedequivalentvalues45 or 60 days after the bahar treatment, which were considerablydifferentfromthoseobservedinthe other treatments. In the combinations, air layers treated with paclobutrazol 45 or 60 days after the bahartreatmentorwithnitrobenzene2.0mllitre^{-1}produced outcomes that were equivalent and had higher $P_N$values than nitrobenzene 2.0 ml litre^{-1}sprayed grafted plants (P_2) did. (Table 1)

AccordingtothedatainTable1,allofthe propagulesatthefloweringstageof*hasthabahar* varied significantly in terms of P_N, whose values intissue-cultureplants(P)($P_1$14.33$molm^{-2}s^{-1}$)and in air layers were comparable but different from those in the grafted plants. Nitrobenzene 2.0 ml litre^{-1}(T)$_6$wasoneofthecompoundsthatproduced resultsthatwerenoticeablygreaterthanthosefrom the other treatments (16.40 mol m^{-2} s^{-1}). Tissue-culture plants (P_1) treated with paclobutrazol or nitrobenzene2.0mllitre^{-1}60daysafterthebahar treatment,orboth,reportedcomparable$P_N$values accordingtothepropaguleandchemicaltreatment combination. During the fruit set stage in *hastha bahar*,neitherthedifferenttypesofpropagulesnor theircombinationswiththechemicalsshowedany significantdifferencesintermsofP_N.Amongthe chemicals,nitrobenzene1.5mllitre^{-1}or2.0ml litre^{-1}plant^{-1}showedcomparablevaluesofP_N significantlydifferentfromthoseseenwithanyof the other chemical treatments. (Table 1)

Regardlessoftheformofpropaguleorseason, we observed a higher rate of photosynthesis, i.e. P_N,duringtheinitialstage(floweringstage),which graduallydeclinedatthefruitsetstage.Thehigher ratewasmostlikelyduetotheplant'sreproductive structures'increaseddemandforassimilates,which isconsistentwithDrogoudi*etal*.,(2012)findings inpomegranate.Inthecurrentstudy,(P_N)washigh inallpropagulesduringfloweringandgradually decreasedduringfruitset,owingtostrongsinks suchasdevelopingfruits,whichdeplete carbohydrates in the source, primarily leaves (Mokhles *etal*., 2019). During flowering, increased rates of photosynthesis were also observed in mango(Laurent*etal*.2008).Increaseddemandfor photosynthatesduringflowerinitiationandgrowth (Wunsche *et al*., 2005) was reported to increase photosynthesis rates in supporting leaves of a branch in apples (Wahl *et al*., 2013), which was also attributed to increased demand from sinks in the form of developing flowers. Treatment with PBZ couldalso haveresultedin a higher photosyntheticrate.TreatmentwithPBZincreases thechlorophyllcontentinwheatandpotato,which isacrucialcomponentforphotosynthesis,andthis may be one mechanism through which PBZ improved photosynthesis in pomegranates (Kishore *etal*.2006;Nouriyani*etal*.2012).Tissueculture plantstreatedwithPBZ alsohad higher transpiration rates and stomatal conductance.

Rateoftranspiration

Atthefloweringstagein*ambebahar*,ascanbe seen from the data presented in Table 2, plants raisedfromdifferenttypesofpropagulesdiffered

Table 1:Rate of photosynthesis (µmol $m^{-2}s^{-1}$) in pomegranate as influenced by different planting materials and chemicals

	Floweringstage								Fruit-set stage							
	ambebahar				*hasthabahar*				*ambebahar*				*hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1	12.76	10.75	10.81	**11.44**	13.68	13.38	12.67	**13.24**	9.59	9.36	11.07	**10.00**	10.25	11.62	12.45	**11.30**
T_2	13.08	11.32	13.47	**12.62**	14.49	12.96	15.65	**14.36**	9.72	9.47	11.66	**10.28**	11.01	11.13	9.69	**10.61**
T_3	13.32	11.61	13.76	**12.89**	13.15	14.07	15.39	**14.20**	9.83	9.92	11.45	**10.40**	9.43	10.67	11.64	**10.58**
T_4	14.15	14.03	9.13	**12.44**	13.86	14.38	14.25	**14.16**	8.74	10.75	11.51	**10.03**	10.26	12.29	13.57	**12.04**
T_5	14.43	14.83	12.87	**14.04**	13.11	13.95	15.52	**14.19**	10.61	12.53	10.53	**10.48**	12.99	13.41	13.79	**13.39**
T_6	14.50	15.46	12.41	**14.12**	17.52	15.29	16.41	**16.40**	11.22	13.22	13.14	**11.74**	14.05	15.43	14.64	**14.71**
T_7	15.10	11.73	11.40	**12.74**	14.30	15.17	13.97	**14.48**	11.32	9.85	12.38	**11.45**	11.09	10.95	10.90	**10.98**
T_8	16.09	12.69	12.88	**13.89**	14.94	12.48	12.86	**13.43**	11.37	10.32	13.07	**12.27**	12.05	11.65	9.03	**10.91**
T_9	17.15	13.16	13.83	**14.71**	16.15	13.98	12.04	**14.06**	11.62	10.61	13.77	**12.87**	9.48	11.74	11.33	**10.85**
T_{10}	11.82	10.92	11.32	**11.35**	12.10	11.69	11.73	**11.84**	8.44	9.06	10.01	**9.17**	9.37	8.54	10.18	**9.37**
Mean	**14.24**	**12.65**	**12.19**		**14.33**	**13.73**	**14.05**		**10.24**	**10.51**	**11.86**		**11.00**	**11.74**	**11.72**	
	P	T	P×T		P	T	P×T		P	T	P×T		P	T	P×T	
SE(m)	0.19	0.36	0.62		0.16	0.30	0.52		0.12	0.23	0.39		0.26	0.47	0.82	
C.D.(5%)	0.56	1.03	1.78		0.47	0.86	1.49		0.35	0.65	1.12		N.S	1.35	N.S	

P1:tissue-culture plants;**P2**:graftedplants;**P3**:Airlayer plants

T1: Methyl jasmonate (MeJA) 100 ppm; **T2**: MeJA 150 ppm; **T3**: MeJA 200 ppm; **T4**: Nitrobenzene (NB) 1.0 ml l^{-1}; **T5**: NB 1.5 ml l^{-1}, **T6**:NB2.0mll^{-1}; **T7**:paclobutrazol(PBZ)0.375gofactiveingredientpermetreofcanopydiameterapplied30daysafterthe‘bahar’treatment;**T8**:sameasT7exceptapplied45 days after the treatment; **T9**: same as T7 except applied 60 days after the treatment; **T10**: Control; sprayed with water after the ‘bahar’treatment

Table 2: Rate of transpiration (mmol $m^{-2}s^{-1}$) in pomegranate as influenced by different planting materials and chemicals

	Flowering stage								Fruit-setstage							
	ambebahar				*hasthabahar*				*ambebahar*				*hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1	6.52	4.57	2.50	**4.53**	3.83	4.89	4.39	**5.10**	3.70	3.95	2.96	**3.65**	2.68	2.89	3.18	**2.91**
T_2	5.21	5.88	2.84	**4.64**	4.28	4.01	4.14	**5.06**	3.82	4.01	3.38	**3.82**	3.23	3.54	2.52	**3.09**
T_3	4.66	5.91	2.98	**4.51**	4.56	3.91	4.52	**5.21**	3.84	4.13	3.48	**3.93**	2.89	2.83	2.76	**2.83**
T_4	7.01	5.59	3.40	**5.33**	3.94	4.17	5.96	**5.42**	3.52	4.61	3.02	**3.48**	3.82	3.1	3.34	**3.42**
T_5	6.45	5.67	3.58	**5.23**	4.70	6.71	5.90	**5.54**	3.57	5.10	3.26	**3.44**	3.27	3.49	3.54	**3.43**
T_6	5.89	6.10	5.47	**5.82**	5.05	6.28	6.95	**5.80**	3.86	5.40	3.51	**4.08**	3.87	4.18	3.8	**3.95**
T_7	7.16	6.55	3.51	**5.74**	4.54	5.07	4.38	**5.93**	3.92	4.02	3.99	**4.29**	3.48	3.66	3.01	**3.38**
T_8	7.85	6.66	3.50	**6.00**	5.07	8.01	5.43	**5.09**	4.02	4.24	4.35	**4.36**	3.47	3.71	2.43	**3.2**
T_9	8.01	7.16	3.52	**6.23**	5.41	7.62	4.80	**5.51**	4.27	4.31	4.59	**4.37**	4.07	3.61	3.26	**3.65**
T_{10}	4.29	3.71	2.48	**3.49**	3.48	4.90	3.73	**4.13**	3.22	3.90	2.85	**3.50**	3.03	2.46	2.48	**2.65**
Mean	**6.30**	**3.38**	**4.30**		**5.56**	**5.02**	**5.28**		**3.77**	**4.37**	**3.54**		**3.38**	**3.35**	**3.03**	
	P	T	P×T		P	T	P×T		P	T	P×T		P	T	P×T	
SE(m)	0.10	0.18	0.31		0.10	0.18	0.32		0.07	0.13	0.23		0.10	0.18	0.32	
C.D.(5%)	0.28	0.52	0.90		0.28	0.52	0.9		0.21	0.39	0.67		0.28	0.52	N.S	

P_1: tissue-cultureplants;**P_2**:graftedplants;**P_3**:Airlayer plants

T_1:Methyljasmonate(MeJA)100ppm;**T_2**:MeJA150ppm;**T_3**:MeJA200ppm;**T_4**:Nitrobenzene(NB)1.0mllitre^{-1};**T_5**:NB1.5mllitre^{-1},**T_6**:NB2.0mllitre^{-1};

T_7: paclobutrazol (PBZ) 0.375 g of active ingredient per metre of canopy diameter applied 30 days after the 'bahar'treatment;**T_8**: same asT_7except applied 45 days after the treatment; **T_9**: same as T_7except applied 60 days after the treatment; **T_{10}**: Control; sprayed with water after the 'bahar'treatment

Table 3: Stomatal conductance (mol m^{-2} s^{-1}) in pomegranate as influenced by different planting materials and chemicals

	Flowering stage								Fruitsetstage							
	ambebahar				*hasthabahar*				*ambebahar*				*hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1	0.25	0.18	0.18	**0.20**	0.29	0.27	0.26	**0.27**	0.19	0.19	0.13	**0.20**	0.14	0.17	0.19	**0.17**
T_2	0.27	0.23	0.21	**0.23**	0.25	0.31	0.24	**0.26**	0.24	0.21	0.14	**0.23**	0.14	0.16	0.17	**0.15**
T_3	0.28	0.27	0.23	**0.26**	0.26	0.24	0.27	**0.26**	0.26	0.24	0.17	**0.25**	0.16	0.12	0.17	**0.15**
T_4	0.34	0.30	0.25	**0.30**	0.23	0.31	0.33	**0.29**	0.21	0.28	0.18	**0.21**	0.17	0.16	0.17	**0.16**
T_5	0.35	0.32	0.27	**0.31**	0.32	0.34	0.36	**0.34**	0.22	0.30	0.21	**0.24**	0.17	0.16	0.23	**0.19**
T_6	0.37	0.34	0.28	**0.33**	0.46	0.40	0.38	**0.41**	0.23	0.32	0.22	**0.24**	0.24	0.26	0.26	**0.25**
T_7	0.42	0.19	0.30	**0.30**	0.28	0.18	0.28	**0.25**	0.26	0.26	0.22	**0.23**	0.16	0.12	0.14	**0.14**
T_8	0.44	0.20	0.31	**0.32**	0.31	0.25	0.26	**0.27**	0.26	0.27	0.25	**0.24**	0.19	0.15	0.13	**0.16**
T_9	0.53	0.24	0.32	**0.36**	0.37	0.24	0.23	**0.28**	0.28	0.29	0.26	**0.25**	0.19	0.14	0.15	**0.16**
T_{10}	0.20	0.16	0.13	**0.16**	0.21	0.21	0.22	**0.21**	0.16	0.21	0.13	**0.17**	0.11	0.12	0.15	**0.13**
Mean	**0.34**	**0.24**	**0.25**		**0.30**	**0.27**	**0.28**		**0.23**	**0.26**	**0.19**		**0.16**	**0.16**	**0.17**	
	P	T	P×T		P	T	P×T		P	T	P×T		P	T	P×T	
SE(m)	0.01	0.01	0.03		0.08	0.01	0.02		0.07	0.01	0.02		0.07	0.01	0.02	
C.D.(5%)	0.02	0.05	0.08		N.S	0.04	0.07		0.01	0.03	0.06		N.S	0.01	N.S	

P_1:Tissue-culture plants;**P_2**: grafted plants;**P_3**:Airlayerplants
T_1:Methyljasmonate(MeJA)100ppm;**T_2**:MeJA 150ppm;**T_3**:MeJA 200ppm;**T_4**:Nitrobenzene(NB)1.0mllitre^{-1};**T_5**:NB1.5mllitre^{-1},**T_6**:NB2.0ml litre^{-1};**T_7**,paclobutrazol(PBZ)0.375gofactiveingredientpermetreofcanopydiameterapplied30daysafterthe'bahar'treatment;**T_8**,sameasT_7exceptapplied 45daysafter thetreatment;**T_9**,same asT_7exceptapplied60 daysafter thetreatment;**T_{10}**,Control; sprayedwith waterafterthe 'bahar'treatment

Table4:Totalchlorophyllcontent(mgg^{-1} freshleaves)inpomegranateasinfluencedbydifferentplantingmaterialsandchemicals

	Floweringstage								**Fruitsetstage**							
	ambebahar				*hasthabahar*				*ambebahar*				*hasthabahar*			
Propagules	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**	**P_1**	**P_2**	**P_3**	**Mean**
T_1	1.37	1.44	1.73	**1.51**	1.75	1.53	1.88	**1.72**	1.54	1.35	1.51	**1.46**	1.37	1.25	1.65	**1.42**
T_2	1.66	1.58	1.95	**1.73**	1.86	1.72	1.52	**1.70**	1.60	1.26	1.60	**1.48**	1.65	1.49	1.29	**1.47**
T_3	1.96	1.65	1.81	**1.81**	1.93	2.63	1.47	**2.01**	1.65	1.16	1.55	**1.45**	1.70	2.29	1.26	**1.75**
T_4	2.36	1.94	1.83	**2.04**	2.82	2.84	2.12	**2.59**	1.68	1.43	1.67	**1.59**	2.55	2.58	1.58	**2.23**
T_5	2.20	2.16	2.15	**2.17**	2.64	3.00	2.49	**2.71**	1.60	1.44	1.68	**1.57**	2.48	2.68	2.10	**2.42**
T_6	2.17	2.23	2.35	**2.25**	3.07	3.24	2.75	**3.02**	1.91	1.54	1.69	**1.71**	2.94	2.95	2.06	**2.65**
T_7	2.40	1.69	1.73	**1.94**	2.04	2.48	1.79	**2.11**	1.96	1.13	1.70	**1.59**	2.04	2.05	1.31	**1.80**
T_8	2.62	1.77	1.60	**1.99**	2.32	2.07	2.45	**2.28**	1.97	1.19	1.80	**1.65**	1.96	1.77	1.19	**1.64**
T_9	3.03	1.81	2.47	**2.43**	3.02	2.57	1.95	**2.51**	2.00	1.40	1.90	**1.76**	2.81	2.14	1.60	**2.18**
T_{10}	1.64	1.19	1.27	**1.36**	1.59	1.57	1.52	**1.56**	1.26	0.92	1.43	**1.20**	1.42	1.30	1.10	**1.27**
Mean	**2.14**	**1.74**	**1.89**		**2.30**	**2.36**	**1.99**		**1.72**	**1.28**	**1.65**		**2.09**	**2.05**	**1.51**	
	P	T	P×T		P	T	P×T		P	T	P×T		P	T	P×T	
SE(m)	0.05	0.1	0.17		0.05	0.10	0.18		0.02	0.04	0.07		0.05	0.09	0.16	
C.D.(5%)	0.15	0.28	0.49		0.16	0.3	0.52		0.06	0.12	N.S		0.14	0.26	0.45	

P_1: tissue-cultureplants;**P_2**:graftedplants;**P_3**:Airlayer plants
T_1:Methyljasmonate(MeJA)100ppm;**T_2**:MeJA150ppm;**T_3**:MeJA200ppm;**T_4**:Nitrobenzene(NB)1.0mllitre^{-1};**T_5**:NB1.5mllitre^{-1},**T_6**:NB2.0mllitre^{-1};
T_7: paclobutrazol (PBZ) 0.375 g of active ingredient per metre of canopy diameter applied 30 days after the 'bahar'treatment;**T_8**: same asT_7except applied 45 days after the treatment; **T_9**: same as T_7except applied 60 days after the treatment; **T_{10}**: Control; sprayed with water after the 'bahar'treatment

significantlyintheirrateoftranspiration(E;mmol $m^{-1}s^{-1}$), with tissue-culture plants (P_1) showing a significantly higher rate (5.78 mmol $m^{-2}s^{-1}$) than that shown by either of the other two propagules. Amongthechemicals,paclobutrazol45daysor60 daysafterthebahartreatmentandnitrobenzene2.0 $mllitre^{-1}$showedcomparableratesoftranspiration. As to the combinationsof propagulesand chemicals, tissue-culture plants treated with paclobutrazol irrespective of the time of its application showed a higher rate of transpiration than that seen in any of the other combinations.

Regardlessofwhenpaclobutrazolwasapplied, grafted plants among the propagules and *ambe bahar*showedhigherratesoftranspirationduring fruitset,whereasamongthecombinations,grafted plantssprayedwithnitrobenzeneat1.5$mllitre^{-1}$or 2.0 ml $litre^{-1}$ recorded higher rates than those in any of the other combinations (Table 2).

The rate of transpirationvariedgreatly according on the propagule at the blooming stage in hastha bahar, with tissue-culture plants and air layers (P_3)displayingsignificantlyhigherratesthan grafted plants. 45 days following the bahar treatment, findingswiththe substances nitrobenzene2.0$mllitre^{-1}$andpaclobutrazolwere comparable.Incomparisontoothercombinations, paclobutrazolgiventotissue-cultureplants45or 60 daysafterthe bahartreatmentcaused transpiration rates to be higher (Table 2).

Duringfruitsetin*hasthabahar*,tissue-culture plantsamongthepropagulesshowedsignificantly higherratesoftranspirationthangraftsorairlayers. Amongthechemicals,nitrobenzeneat1.5$mllitre^{-1}$or2.0$mllitre^{-1}$showedcomparableresults.Asto the combinations,noneof themdiffered significantly from any of the rest in terms of the rate of transpiration (Table 2).

Stomatalconductance

Stomatal conductance (g_s) varied significantly acrossthepropagulesatthebloomingstagein*ambe bahar*(Table 3), with tissue-cultureplants exhibitinghigher$g_s$thanairlayersorgrafts.High g_s,whichwascomparabletoeitherofthetwodoses ofnitrobenzene(1.5$mllitre^{-1}$or2.0$mllitre^{-1}$),was observedinplantstreatedwithpaclobutrazol45or 60daysafterthebahartreatment.Paclobutrazol-treatedtissue-cultureplantsshowedhighgsamong thecombinations60daysafterthebahartreatment.

Thepropagulesvariedgreatlythroughoutfruit set in *ambe bahar*, with grafts displaying greater valuesthaneithertissue-cultureplantsorairlayers. Amongthecompounds,methyljasmonate150ppm and paclobutrazol, regardless of the date of administration, demonstrated noticeably higher stomatalconductancethantheothertreatments.In comparison to the other combinations, the one containing nitrobenzene, regardless of dose, or paclobutrazol60daysafterthebahartreatmentand grafted plants displayed increasedstomatal conductance (Table 3).

Grafts displayed greater values than either tissue-cultureplantsorairlayersduringfruitsetin *ambe bahar*, where propagules varied greatly in termsofg_s.Paclobutrazol,regardlessofthedateit wasapplied,andmethyljasmonate150ppmamong the compounds had noticeably higher stomatal conductivitythan the other treatments.The combinationswithnitrobenzene,regardlessofdose, orpaclobutrazol60daysafterthebahartreatment andgraftedplantshadgreaterstomatalconductance than the others (Table 3).

The propagules and the combinationsof propagules and chemicals did not exhibit any discernible variations in conductance during fruit settingin*hasthabahar*.Butwhencomparedtothe otherchemicaltreatments,nitrobenzene2.0$mllitre^{-1}$ displayed noticeably higher values (Table 3).

Plants treated with PBZ have also shown increasedtranspirationratesinpreviousstudies.In peonies,PBZapplicationincreasedphotosynthetic rateandtranspirationratesignificantly(Xing*etal.*, 2018).Photosynthesis has been studied as a significant limitation due to stomatal opening. In peony, both transpirationrate and stomatal conductancewerefoundtobehigherinPBZtreated plantsthanincontrolplants,similartoourfindings. Another research by Berova and Zlatev (2003) whichshowedahightranspirationrateandstomatal conductance after PBZ treatment was also in agreementwithourfindings.Thismaybeexplained by the fact that a large stomatal opening and conductance are conducive to CO_2 entry into the intracellular space, allowing PBZ to improve photosynthesis.

Totalchlorophyllcontent

The datain Table 4 show thatall three propagules significantly influenced the total chlorophyllcontentofleavesatthefloweringstage of*ambebahar*,withtissue-cultureplantsshowing the highest levels (2.14 mg g^{-1}), which were significantly higher than those in the other two kinds of propagules. Paclobutrazol, applied 60 days afterthebahartreatment,andnitrobenzene,applied to1.5mllitre^{-1}or2.0mllitre^{-1}plants,respectively, bothreported chemical treatmentswith significantlygreatertotalchlorophyllcontentsthan any of the others. When compared to the other combinations, tissue-culture plants treated with paclobutrazol 45 or 60 days following the bahar treatment displayed the highest levels of total chlorophyll.

The total chlorophyll content of leaves varied significantly between all propagules during fruit setstageof*ambebahar*;tissue-cultureplantshad the highest levels (1.72 mg g^{-1}), and the highest chemicalconcentrationswerefoundfor paclobutrazol 45 or 60 days after the bahar treatment and nitrobenzene at 2.0 ml litre^{-1}. However, none of the combinations really stood out from the others (Table 4).

Atthefloweringstagein*hasthabahar*,eachof thethreepropagulediffersignificantlyfromthe othertwointermsofchlorophyllcontent,withthe graftedplantsshowingthehighestlevels.Among the chemicals, nitrobenzene at 2.0 ml litre^{-1}led to significantly higher levels than those seen in any of the other chemical treatments.The combinations alsodifferedsignificantlyamongthemselves,with thecombinationofnitrobenzeneatanyofthethree dosesandgraftedplantsrecordingthehighestlevels of chlorophyll (Table 4).

During fruit set in *hastha bahar*, both tissue-cultureplantsand graftedplantsshowed significantly higher levels of chlorophyll; among the chemicals, significantly higher levels of chlorophyll were seen in plants treated with nitrobenzene at 2.0 ml litre^{-1} than those in any of theothertreatments;amongthecombinations,that ofgraftedplantsandnitrobenzene,whetherat1.0 mllitre^{-1}or1.5mllitre^{-1}or2.0mllitre^{-1},recorded thehighestlevelsoftotalchlorophyll,significantly higherthanthoseinanyoftheothercombinations (Table 4).

Chlorophyllcontentwashighintissueculture and grafted plants treated with paclobutrazol or nitrobenzene at the flowering stage in both *ambe bahar* and *hastha bahar*.

Chlorophyllplaysadualroleinphotosynthesis as an essential componentof the primary photosynthetic reaction. It captures light and also actsasamediumforchargeseparationandelectron transport caused by light (Zhao *et al*., 2011). Paclobutrazol had an important impact on the biosynthesis of chloroplast pigments.The increased chlorophyll content in paclobutrazol-treated plants may be due to reduced reactive oxygen damage and improvements in carotenoids, ascorbate, and ascorbate peroxidase levels. Plants treated with paclobutrazol synthesized more cytokinin, which enhanced chloroplastdifferentiationand chlorophyllbiosynthesis,and prevented chlorophyll degradation, according to Niveditha DeviandSomasundaram(2012).Whencompared to untreated controls,datasuggestedthat Pomegranate plants treated with plant growth regulators,especiallyPBZ,producedmorefruitand had improved photosynthetic characteristics.

Conflictofinterest

The authors declare that the research was conducted in the absence of any commercial or financialrelationshipsthatcouldbeconstruedasa potential conflict of interest.

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