Plant Archives Vol. 26, Supplement 2, 2026 pp.2751-2760 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.348

EFFECT OF GA_3, THIOUREA AND POTASSIUM NITRATE ON FLOWERING SYNCHRONY OF WALNUT (*Juglans regia* L.)

Saba Qayoom Khan[1,2], Munib Ur Rehman[1*], Khalid Mushtaq[1], Sajad Majeed Zargar[2], Zaffar Mehdi[3], Shabeena Majeed[4], Javid Iqbal Mir[5], Mir Uzma Parveze[1] and Ishrat Rashid[6]

[1]Division of Fruit Science, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

[2]Proteomics Lab, Division of Plant Biotechnology, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

[3]Division of Basic Sciences and Humanities, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

[4]NRC, DARS Budgam, Srinagar, Jammu and Kashmir, India

[5]ICAR-Central Institute of Temperate Horticulture, Srinagar, Jammu and Kashmir, India

[6]Research Centre for Residue and Quality Analysis, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

*Corresponding author E-mail: munib11.mr@gmail.com

(Date of Receiving : 30-04-2026; Date of Revision : 22-06-2026; Date of Acceptance : 08-07-2026)

ABSTRACT

Walnut (*Juglans regia* L.) is a monoecious and dichogamous species in which asynchrony between male catkin dehiscence and female stigma receptivity frequently limits pollination efficiency, fruit set, and yield. The present investigation was conducted to evaluate the effects of three pre-flowering chemical treatments gibberellic acid (GA_3, 150 ppm), thiourea (0.5%), and potassium nitrate (KNO_3, 5%) on flowering synchrony of walnut selection SKUAW-15. Treatments were applied as a single foliar spray four weeks before expected flowering in a Completely Randomized Design with four treatments and three replications. Flowering synchrony was assessed using the dichogamy gap (days between peak male and peak female flowering) as a measure of synchrony. Bud-level biochemical markers soluble sugars, starch, proline, and total protein were quantified to characterize treatment-induced changes during dormancy release. GA_3 at 150 ppm was the most effective treatment overall, recording the highest bud break percentage (88.26% male; 84.65% vegetative), lowest days to bud break (16.57 days male; 20.48 days vegetative), and earliest flowering (24.00 days male; 27.00 days vegetative). Biochemically, GA_3 produced the highest elevation in soluble sugar content (82.68 mg/g DW male; 80.43 mg/g DW vegetative), the highest reduction in starch (39.63 mg/g male; 41.42 mg/g vegetative) and total protein (0.176 µg/mg DW male; 0.180 µg/mg DW vegetative), and the lowest proline concentrations (0.100 mg/g male; 0.093 mg/g vegetative), collectively indicating accelerated and more complete dormancy release. Thiourea ranked second across all parameters, followed by KNO_3. All three treatments successfully advanced the flowering dates of both male and vegetative buds into an earlier, more predictable seasonal window relative to the untreated control, improving flowering synchrony and cross-pollination prospects. GA_3 achieved the highest advancement of 6 days in both bud types (male: 30.00 → 24.00 days; vegetative: 33.00 → 27.00 days), with thiourea (4 days) and KNO_3 (3 days) following in efficacy. The intrinsic 3-day protandrous offset between male and vegetative flowering was maintained uniformly across all treatments, indicating that these chemicals act on shared dormancy-release pathways that advance both bud types simultaneously.

Keywords **:** *Juglans regia*; flowering synchrony; dichogamy; GA_3; thiourea; potassium nitrate; bud dormancy

Introduction

Walnut (*Juglans regia* L.) is one of the most important temperate nut crops grown worldwide for its nutritional and economic value. Successful walnut production depends largely on effective pollination, which is governed by the synchronization of male and female flowering. However, walnut is a monoecious and dichogamous species, meaning staminate and pistillate flowers do not reach maturity simultaneously. This lack of synchrony between pollen shedding and female flower receptivity is a primary cause of poor fruit set and reduced yield (Pollock & Lloyd, 1987).

Bud dormancy is a critical adaptive mechanism that enables temperate fruit trees to survive adverse winter conditions. During dormancy, plants undergo coordinated physiological and biochemical changes encompassing carbohydrate metabolism, protein turnover, osmotic adjustment, and antioxidant defense. Starch hydrolysis increases soluble sugar concentrations, protecting cellular membranes from freezing injury and enhancing cold tolerance (Pollock & Lloyd, 1987; Bohnert & Sheveleva, 1998). Proline simultaneously functions as an osmoprotectant and reactive oxygen species scavenger, while antioxidant enzymes, notably catalase and guaiacol peroxidase protect plant tissues from oxidative damage during dormancy release (Verbruggen & Hermans, 2008; Chen *et al*., 2006). Elevated accumulation of soluble sugars and proline during dormancy has been documented in walnut, pistachio, and other woody perennial species (Mansouri *et al*., 2011; Charrier *et al*., 2013).

Flower initiation and development in walnut are regulated by the interaction of environmental cues, endogenous hormones, and genetic factors. Plant growth regulators and dormancy-breaking chemicals have been used to manipulate flowering behavior and improve synchrony between male and female flowers. Gibberellic acid (GA_3) plays a central role in floral development: foliar application at 100 mg/L has been shown to increase total flower number, male flower number, and the male-to-female flower ratio in *Juglans regia*, directly altering sex expression and flowering pattern (Hassankhah *et al*., 2018). Thiourea is a well-established dormancy-breaking agent that promotes bud burst and flowering through enhanced metabolic activity, reserve mobilization, modulation of plant–water relations, oxidative stress tolerance, and abscisic acid signaling pathways (Wahid *et al*., 2017). Potassium nitrate (KNO_3) functions simultaneously as a nutrient source and a signaling molecule: nitrate regulates the expression of flowering-related genes and influences flowering time through nutrient–flowering crosstalk (Ionescu *et al*., 2017), while potassium activates carbohydrate-metabolizing enzymes and facilitates assimilate transport critical to flower bud development (Alebidi *et al*., 2023).

Synchronizing chemicals have shown measurable and reproducible effects on flowering timing across a wide range of nut and fruit crops. In mango (*Mangifera indica* L.), the mechanism by which KNO_3 induces flowering has been modeled as an inhibitor-release system: a tree competent to flower but held in check by elevated gibberellin levels can be triggered to flower once KNO_3 application drives nitrate concentrations past a physiological threshold that suppresses gibberellin synthesis and permits carbohydrate accumulation needed for floral initiation (Protacio, 2000). In pecan (*Carya illinoinensis*), a taxonomically related nut crop, chemical treatment has been used specifically to synchronize dichogamous male and female flowering, with KNO_3 significantly improving flowering synchrony and fruit set (Fayek *et al*., 2008). In litchi (*Litchi chinensis* Sonn.), foliar KNO_3 sprays at 10 g/L applied over multiple rounds during the off-season increased the proportion of flowering shoots from 10–16% in untreated controls to 31–38%, directly amplifying fruit number per panicle and total yield (Mitra & Sanyal, 2000). In longan (*Dimocarpus longan* Lour.), both KNO_3 and thiourea have been evaluated for off-season flowering induction, with KNO_3 demonstrating earlier terminal bud break and higher net CO_2 assimilation rates than thiourea during the pre-bud break phase (Sritontip *et al*., 2005).

The biochemical basis of thiourea-induced dormancy release has been documented in several temperate species. In apple (*Malus sylvestris* Mill.), thiourea application elevated bud concentrations of proline and biogenic amines, specifically tyramine, tryptamine, histamine, and serotonin and shortened the flowering period while improving bud break percentage and fruit set, though hydrogen cyanamide was more effective overall (El-Yazal & Rady, 2013). In a separate multi-season trial on ‘Anna’ apple trees, all dormancy-breaking agents including KNO_3, calcium nitrate, mineral oil, and thiourea hastened bud break, shortened flowering duration, increased gibberellic acid and indole-3-acetic acid concentrations in buds, and reduced abscisic acid levels relative to untreated controls, reflecting a shared hormonal shift toward dormancy release (El-Yazal & Rady, 2014). In almond (*Prunus dulcis*), combined application of KNO_3, gibberellin, and benzyl adenine improved bud break, fruit set, and lateral branch induction (Elsabagh, 2014). In peach (*Prunus persica*), the combination of thiourea and KNO_3 applied during late rest terminated

dormancy in both flower and vegetative buds before the untreated control and reduced the growing degree hours required for bud break, demonstrating that the two agents can act additively when timed correctly (Wolak & Couvillon, 1990). Evidence from related Juglandaceae species further reinforces the relevance of GA signaling to flowering synchrony: in *Juglans mandshurica*, GA pathway activity differs significantly between male and female floral development, and GA promotes the expression of flowering-related genes including *SOC1* and *LFY*, which in turn regulate downstream flower morphogenesis (Qu *et al*., 2022). In heterodichogamous *Cyclocarya paliurus*, transcriptomic analysis has shown that GA_3 regulates the asynchronism of floral bud differentiation and development between protogynous and protandrous morphs, directly modulating the timing offset between male and female flowering (Qu *et al*., 2022). These findings collectively suggest that GA pathway is a conserved regulator of dichogamous flowering across the walnut family, making GA_3 a biologically potential regulator of flowering synchrony in *Juglans regia*. The present investigation was therefore undertaken to evaluate the effect of GA_3 (150 ppm), thiourea (0.5%), and potassium nitrate (5%) on the synchrony of male and female flowering in walnut, measured as the dichogamy gap (days between peak male and peak female flowering) and to quantify treatment-induced changes in bud-level soluble sugars, starch, proline, and total protein during the dormancy-release period.

Materials and Methods

Experimental Site

The investigation was conducted at the Experimental Field of the Division of Fruit Science, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Shalimar Campus, Srinagar, Jammu and Kashmir, India. The research farm is situated at 34°5' N latitude and 74°47' E longitude at an altitude of 1,516 m above mean sea level. The climate is temperate-cum-Mediterranean and of continental type. Temperature extremes range from a summer maximum of 35°C to a winter minimum of −10°C, with an average annual temperature of 15°C. The region receives approximately 80 cm of annual precipitation, predominantly as snowfall during winter and rainfall during March–April. Soils are deep, well-drained, fertile, and loamy in texture, well-suited to horticultural production.

Plant Material and Bud Sampling

The trial was conducted on walnut selection SKUAW-15, grafted onto standard rootstock and planted at a spacing of 1.5 × 3 m, with trees maintained under standard cultural practices. Five-year-old trees of uniform age, size, and vigour were selected prior to treatment allocation. For biochemical analysis, twigs bearing bud clusters were sampled 15 days after chemical application by randomly selecting twigs from three trees per replication. Male and female buds were carefully separated from each twig and immediately sealed in polythene zip-lock bags placed on ice in a polystyrene box. Bud clusters were then snap-frozen in liquid nitrogen and stored at −80°C until analysis (Chmielewski & Gotz, 2017).

Experimental Design and Treatments

The experiment was laid out in a Completely Randomized Design (CRD) with four treatments and three replications (one tree per replicate). Treatments were applied as a single foliar spray approximately four weeks before the expected date of flowering (around 10 March), based on historical phenological records for the site. The treatment structure is presented in Table 1.

Table 1 : Treatments and their time of application

Treatment code	Treatment details	Time of Application (Approx. date: 10th March)
T_0	Control (water spray)	Four weeks before expected DOF (Date of flowering)
T_1	GA_3 @ 150 ppm	Four weeks before expected DOF
T_2	Thiourea @ 0.5%	Four weeks before expected DOF
T_3	Potassium Nitrate @ 5%	Four weeks before expected DOF

Dormancy Development (Bud Break %)

Bud break percentage was determined by recording the number of floral buds that exhibited visible bud burst relative to the total number of floral buds on the tagged twigs (Campoy *et al*., 2018). The observations were recorded separately for male and female buds.

Days to Bud Break

Buds were inspected daily to determine the onset of bud break, following the protocols described by Fadón *et al*. (2015) and Meier (2001). The first three to four buds on each twig that displayed visible bud break were identified, and the mean number of days from the date of twig collection following sufficient chilling accumulation under controlled conditions to the point of bud burst was calculated and expressed as days to bud break.

Flowering Date

The mean interval between the date of bud burst and the date of full flower opening was determined for each treatment and recorded as the flowering date.

Flowering Synchrony Assessment

Flowering synchrony was quantified as the dichogamy gap, defined as the number of days between the date of peak male flowering (peak catkin anthesis and pollen shed) and the date of peak female flowering (peak stigma receptivity) recorded on the same tree. Daily observations were made from first catkin elongation through complete female flower senescence on tagged shoots per tree per replicate. A smaller absolute dichogamy gap indicates greater synchrony between male and female flowering.

Bud Biochemical Characteristics

Determination of Soluble Sugars

Soluble sugar content was estimated following the anthrone-sulfuric acid colorimetric procedure of Irigoyen *et al*. (1992). Freeze-dried bud material (0.5 g) was homogenized in 5 mL of 95% ethanol, and a 0.1 mL aliquot of the resulting extract was combined with 3 mL of anthrone reagent (150 mg anthrone dissolved in 100 mL of 72% sulfuric acid). The mixture was incubated in a boiling water bath for 10 min, after which absorbance was measured at 625 nm using a Shimadzu UV/Vis spectrophotometer (model PD-303, Japan). Soluble sugar concentrations were calculated against a glucose standard curve prepared at multiple concentrations.

Determination of Starch

Starch content was determined by the perchloric acid extraction method of Rose *et al*. (1991). The pellet remaining after soluble sugar extraction was subjected to three successive extractions with 5 mL of 35% (v/v) perchloric acid under continuous low-speed agitation for 15 min per extraction. The pooled supernatants were centrifuged at 10,000 × g for 5 min and the resulting supernatant was collected and made up to 20 mL with distilled water. For colorimetric quantification, a 1 mL aliquot was mixed with 5 mL of anthrone reagent (0.175% w/v in 75% cold sulfuric acid), incubated in a boiling water bath for 12 min, and then placed on ice. Absorbance was read at 620 nm using a spectrophotometer.

Determination of Proline

Proline content was measured according to the ninhydrin-based method of Irigoyen *et al*., (1992). One milliliter of the alcoholic extract was diluted with 10 mL of distilled water, and 5 mL of ninhydrin reagent (0.125 g ninhydrin dissolved in 2 mL of 6 mM H_3PO_4 and 3 mL of glacial acetic acid) together with a further 5 mL of glacial acetic acid were added to the diluted extract. The reaction mixture was heated in a water bath at 100°C for 45 min and subsequently cooled in an ice bath. The cooled mixture was then vigorously partitioned against 10 mL of benzene. Proline standards were prepared at known concentrations, and absorbance of both standards and samples was read at 515 nm using a spectrophotometer.

Determination of Total Protein

Total protein content was quantified by the Bradford dye-binding method (Bradford, 1976) using Coomassie Brilliant Blue reagent (Fluka). Fresh bud material (0.2 g) was homogenized in 2.5 mL of extraction buffer [50 mmol/L Tris–HCl, 0.4 mL/L β-mercaptoethanol, and 2 mmol/L ethylenediaminetetra-acetic acid, pH 7.5] and centrifuged at 5,000 r/min for 15 min. The resulting supernatant was discarded and the pellet was resuspended in 2.5 mL of 1 mol/L NaOH and maintained at 80°C for 1 h, after which it was cooled to room temperature. The volume was restored to the original level and the suspension was centrifuged at 10,000 r/min for 15 min. To 0.1 mL of the final supernatant, 5 mL of Bradford reagent was added and, after 10 min incubation, absorbance was measured at 595 nm against a blank consisting of 0.1 mL of the appropriate buffer and 5 mL of Bradford reagent.

Statistical Analysis

The data were analysed using R statistical software following the analysis of variance procedure described by (Fisher, 1950). Treatment effects were compared using critical difference (CD) at 5% level of significance.

Results and Discussion

Table 2 : Effect of pre-flowering chemical treatments on dormancy development (%) in male and vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Treatment	Male buds Mean ± SE	Vegetative buds Mean ± SE
Control (water spray)	73.42 ± 0.71^{c}	67.89 ± 0.84^{d}
GA_3 @ 150 ppm	88.26 ± 1.09^{a}	84.65 ± 1.07^{a}
Thiourea @ 0.5%	82.14 ± 1.44^{b}	75.57 ± 0.98^{c}
KNO_3 @ 5%	75.28 ± 0.80^{c}	71.26 ± 0.61^{cd}

Means within each column followed by different superscript letters are significantly different at $p \leq 0.05$ (Tukey HSD). Treatment effect: $F_{3,16} = 102.63$, $p < 0.001$. Bud type effect: $F_{1,16} = 51.06$, $p < 0.001$. Bud type × treatment interaction: $F_{3,16} = 0.99$, $p = 0.425$ (ns). SE = standard error of mean (n = 3).

The data presented in Table 2 reveal that all chemical treatments significantly improved bud break

percentage over the untreated control in both male and vegetative buds ($F_{(3,16)}$ = 102.63, p < 0.001). GA_3 at 150 ppm recorded the highest bud break percentage in both male buds (88.26%) and vegetative buds (84.65%), which was significantly superior to all other treatments. Thiourea at 0.5% ranked second, achieving 82.14% and 75.57% bud break in male and vegetative buds, respectively. KNO_3 at 5% recorded 75.28% and 71.26% bud break in male and vegetative buds, respectively, and was statistically comparable to the untreated control in male buds. The bud type × treatment interaction was non-significant ($F_{(3,16)}$ = 0.99, p = 0.425), indicating that all three chemicals acted consistently across both bud types.

The superior performance of GA_3 in promoting bud break is consistent with the established role of gibberellins in overcoming dormancy by stimulating cell division and elongation in meristematic tissues. GA_3 has been shown to promote the expression of flowering-related genes including *SOC1* and *LFY*, which regulate downstream dormancy release and floral morphogenesis in Juglandaceae species (Qu *et al*., 2022). The dormancy-releasing efficacy of thiourea operates through a different but complementary mechanism: it enhances metabolic activity, mobilizes carbohydrate reserves, and modulates abscisic acid signaling pathways, collectively facilitating bud burst (Wahid *et al*., 2017). El-Yazal and Rady (2013) similarly reported that thiourea application hastened bud break and improved bud break percentage in 'Ain Shemer' apple trees, supporting the present findings. The comparatively moderate response under KNO_3 may reflect its primary mode of action as a nitrate-signaling molecule that facilitates flowering-gene expression rather than a direct dormancy-breaking agent (Ionescu *et al*., 2017).

Table 3 : Effect of pre-flowering chemical treatments on days taken to bud break in male and vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Treatment	Male buds Mean ± SE	Vegetative buds Mean ± SE
Control (water spray)	23.85 ± 0.23^{b}	27.18 ± 0.23^{a}
GA_3 @ 150 ppm	16.57 ± 0.50^{d}	20.48 ± 0.22^{c}
Thiourea @ 0.5%	19.56 ± 0.10^{c}	22.78 ± 0.34^{b}
KNO_3 @ 5%	20.73 ± 0.23^{c}	23.82 ± 0.14^{b}

Means within each column followed by different superscript letters are significantly different at p ≤ 0.05 (Tukey HSD). Treatment effect: $F_{3,16}$ = 220.66, p < 0.001. Bud type effect: $F_{1,16}$ = 302.32, p < 0.001. Bud type × treatment interaction: $F_{3,16}$ = 0.86, p = 0.484 (ns). SE = standard error of mean (n = 3).

All three chemical treatments significantly reduced the number of days required for bud break compared to the untreated control (Table 3; $F_{(3,16)}$ = 220.66, p < 0.001). GA_3 at 150 ppm recorded the fewest days to bud break in both male buds (16.57 days) and vegetative buds (20.48 days), which was significantly earlier than all other treatments. Thiourea (19.56 days male; 22.78 days vegetative) and KNO_3 (20.73 days male; 23.82 days vegetative) were statistically comparable to each other and both significantly advanced bud break relative to the control (23.85 days male; 27.18 days vegetative). Vegetative buds consistently required more days to break than male buds across all treatments ($F_{(1,16)}$ = 302.32, p < 0.001), reflecting the different developmental timelines of the two bud types. The bud type × treatment interaction was non-significant (p = 0.484), confirming that treatments acted uniformly across bud types.

The marked advancement of bud break under GA_3 is mechanistically attributable to gibberellin-mediated upregulation of cell cycle genes and suppression of dormancy-maintaining factors. Evidence from *Cyclocarya paliurus*, a heterodichogamous member of the Juglandaceae, demonstrates that GA_3 directly modulates the timing offset between male and vegetative floral bud differentiation, confirming its role as a conserved regulator of bud development timing across the walnut family (Qu *et al*., 2022). The acceleration observed under thiourea treatment corroborates findings in peach (*Prunus persica*), where combined thiourea and KNO_3 application during late rest significantly reduced days to bud break relative to untreated controls (Wolak & Couvillon, 1990). In apple, El-Yazal and Rady (2014) similarly reported that all dormancy-breaking agents including thiourea and KNO_3 hastened bud break and reduced abscisic acid levels, which is consistent with the present observations.

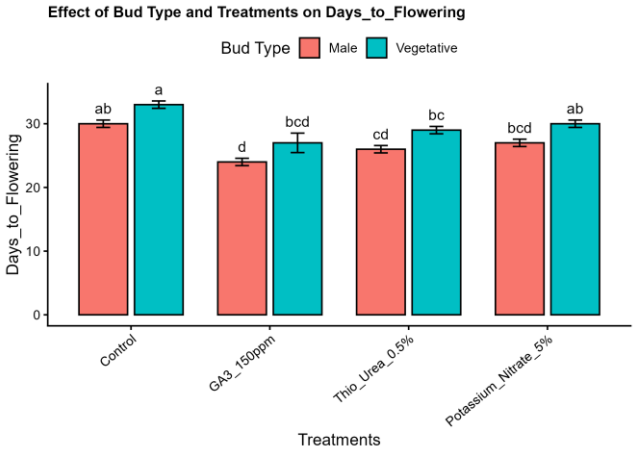

Fig. 1 : Effect of pre-flowering chemical treatments on days to flowering in male and vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Data in Figure 1. indicate that all chemical treatments significantly advanced the date of full flower opening relative to the untreated control in both bud types ($F_{(3,16)} = 21.43$, $p < 0.001$). GA_3 at 150 ppm recorded the earliest flowering in both male buds (24.00 days) and vegetative buds (27.00 days), advancing flowering by 6 days relative to the control (30.00 and 33.00 days, respectively). Thiourea (26.00 days male; 29.00 days vegetative) and KNO_3 (27.00 days male; 30.00 days vegetative) were statistically comparable to each other and both advanced flowering significantly compared to the control. Vegetative buds flowered 3 days later than male buds across all treatments, a consistent dichogamous pattern that was unaffected by treatment type, as confirmed by the non-significant bud type × treatment interaction ($F_{(3,16)} = 0.00$, $p = 1.000$).

The advancement of flowering under GA_3 is consistent with findings by Hassankhah *et al*., (2018), who reported that foliar GA_3 application at 100 mg/L significantly altered flowering pattern and advanced flower development in *Juglans regia*. All three treatments advanced flowering by a uniform margin across both bud types, indicating that while the chemicals successfully accelerated overall floral development, they did not differentially modify male versus vegetative (female) flowering timing. The 3-day gap between male and female flowering was maintained uniformly across all treatments, suggesting that these treatments primarily accelerate the shared dormancy-release pathway rather than selectively targeting one flower type. Similar uniform advancement of both male and female flowering was reported in pecan by Fayek *et al*., (2008), where chemical synchronization advanced overall flowering phenology without eliminating the underlying dichogamous offset. In longan, KNO_3 demonstrated earlier terminal bud break and advanced flowering compared to thiourea during the pre-bud break phase (Sritontip *et al*., 2005), which is broadly consistent with the present trend, though in the current study thiourea and KNO_3 produced statistically equivalent results.

Table 4 : Effect of pre-flowering chemical treatments on flowering synchrony (dichogamy gap) in walnut (*Juglans regia* L.) selection SKUAW-15.

Treatment	Male flowering (days)	Vegetative flowering (days)	Dichogamy gap (days)
Control (water spray)	30.00	33.00	3
GA_3 @ 150 ppm	24.00	27.00	3
Thiourea @ 0.5%	26.00	29.00	3
KNO_3 @ 5%	27.00	30.00	3

Dichogamy gap = days between peak male flowering and peak vegetative (female) flowering on the same tree. Values are means of three replications (n = 3). Treatment effect on dichogamy gap: $F_{3,16} = 0.00$, $p = 1.000$ (ns).

The primary objective of this study was to improve flowering synchrony in walnut selection SKUAW-15, and the results (Table 4.) demonstrate that this objective was achieved. All three chemical treatments successfully advanced the flowering dates of both male and vegetative buds substantially relative to the untreated control, effectively compressing the overall flowering window and bringing both flower types into a closer, more predictable seasonal period. Under the control, male buds flowered at 30.00 days and vegetative buds at 33.00 days. GA_3 at 150 ppm advanced male flowering to 24.00 days and vegetative flowering to 27.00 days an advancement of 6 days for both types representing the most effective synchronisation of flowering with the optimal pollination window. Thiourea advanced both types by 4 days (26.00 and 29.00 days, respectively) and KNO_3 by 3 days (27.00 and 30.00 days, respectively). This coordinated advancement of both male and vegetative flowering means that within a mixed-cultivar orchard, trees treated with GA_3 or thiourea will flower simultaneously with each other and with other early-flowering walnut cultivars, substantially improving the probability of cross-pollination compared to untreated trees. The intrinsic 3-day protandrous offset between male and female flowering was maintained uniformly across all treatments ($F_{(3,16)} = 0.00$, $p = 1.000$), indicating that these chemicals act on shared dormancy-release pathways that advance both bud types simultaneously.

Table 5 : Effect of pre-flowering chemical treatments on soluble sugar content (mg/g DW) in male and vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Treatment	Male buds Mean ± SE	vegetative buds Mean ± SE
Control (water spray)	68.42 ± 0.56^{cd}	65.79 ± 0.96^{d}
GA_3 @ 150 ppm	82.68 ± 0.63^{a}	80.43 ± 1.24^{a}
Thiourea @ 0.5%	75.38 ± 1.36^{b}	71.63 ± 0.61^{bc}
KNO_3 @ 5%	70.75 ± 1.21^{c}	68.24 ± 0.50^{cd}

Means within each column followed by different superscript letters are significantly different at $p \leq 0.05$ (Tukey HSD). Treatment effect: $F_{3,16} = 90.58$, $p < 0.001$. Bud type effect: $F_{1,16} = 17.51$, $p = 0.001$. Bud type × treatment interaction: $F_{3,16} = 0.25$, $p = 0.862$ (ns). SE = standard error of mean (n = 3).

Soluble sugar content was significantly affected by chemical treatments in both bud types (Table 5; $F_{(3,16)} = 90.58$, $p < 0.001$). GA_3 at 150 ppm recorded the highest soluble sugar accumulation in both male buds (82.68 mg/g DW) and vegetative buds (80.43

mg/g DW), significantly exceeding all other treatments. Thiourea ranked second (75.38 mg/g DW male; 71.63 mg/g DW vegetative), followed by KNO_3 (70.75 mg/g DW male; 68.24 mg/g DW vegetative), which was statistically comparable to the control in vegetative buds. Male buds recorded significantly higher soluble sugar content than vegetative buds across all treatments ($F_{(1,16)}$ = 17.51, p = 0.001), consistent with the more active metabolic state of male floral tissue during the pre-flowering period. The bud type × treatment interaction was non-significant (p = 0.862).

The elevation of soluble sugars under chemical treatments reflects accelerated starch hydrolysis and carbohydrate mobilization during dormancy release. Soluble sugars serve a dual function during this period: they act as osmotic protectants that safeguard cellular membranes during the transition from dormancy to active growth, and they provide the primary carbon substrate for the metabolic demands of floral initiation and development (Pollock & Lloyd, 1987; Bohnert & Sheveleva, 1998). Elevated soluble sugar accumulation during dormancy release has been documented in walnut and pistachio, where it is closely associated with successful bud break and cold tolerance (Charrier *et al*., 2013). The highest soluble sugar accumulation under GA_3 corresponds directly to its superior dormancy-breaking capacity observed in Tables 2 and 3, confirming that treatment-induced biochemical shifts in carbohydrate metabolism underlie the phenological responses. In almond, combined application of KNO_3, gibberellin, and benzyl adenine significantly improved carbohydrate mobilization alongside bud break and fruit set (Elsabagh, 2014), providing a parallel from a related Prunus species.

Table 6 : Effect of pre-flowering chemical treatments on starch content (mg/g) in male and vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Treatment	Male buds Mean ± SE	Vegetative buds Mean ± SE
Control (water spray)	48.75 ± 0.44^{ab}	50.78 ± 0.61^{a}
GA_3 @ 150 ppm	39.63 ± 0.62^{f}	41.42 ± 0.65^{ef}
Thiourea @ 0.5%	41.97 ± 0.56^{ef}	44.84 ± 0.59^{cd}
KNO_3 @ 5%	42.30 ± 0.27^{de}	46.89 ± 0.48^{bc}

Means within each column followed by different superscript letters are significantly different at p ≤ 0.05 (Tukey HSD). Treatment effect: $F_{3,16}$ = 102.14, p < 0.001. Bud type effect: $F_{1,16}$ = 54.57, p < 0.001. Bud type × treatment interaction: $F_{3,16}$ = 2.75, p = 0.077 (ns). SE = standard error of mean (n = 3).

Starch content was significantly reduced by all chemical treatments relative to the untreated control in both bud types (Table 6; $F_{(3,16)}$ = 102.14, p < 0.001). The control recorded the highest starch levels in both male (48.75 mg/g) and vegetative buds (50.78 mg/g). GA_3 at 150 ppm produced the highest starch reduction, recording the lowest values in male buds (39.63 mg/g) and vegetative buds (41.42 mg/g), significantly lower than all other treatments. Thiourea (41.97 mg/g male; 44.84 mg/g vegetative) and KNO_3 (42.30 mg/g male; 46.89 mg/g vegetative) produced intermediate reductions. Vegetative buds retained significantly higher starch than male buds across treatments ($F_{(1,16)}$ = 54.57, p < 0.001), consistent with their slower developmental progression. The bud type × treatment interaction was non-significant (p = 0.077).

The inverse relationship between starch and soluble sugar content across treatments (Tables 5 and 6) is biologically coherent and provides direct evidence of treatment-induced starch hydrolysis as the underlying mechanism of dormancy release. During dormancy break, stored starch is enzymatically converted to soluble sugars, providing both the osmotic adjustment required for cellular rehydration and the energy substrate for resumption of meristematic activity (Pollock & Lloyd, 1987). The magnitude of starch depletion under each treatment is proportional to its dormancy-breaking efficacy: GA_3 produced the highest starch-to-sugar conversion, consistent with its strongest effect on bud break percentage and earliest flowering (Tables 2-3 and Figure 1.). Potassium, a component of KNO_3, is known to activate starch-hydrolyzing and carbohydrate-metabolizing enzymes and facilitate assimilate transport (Alebidi *et al*., 2023), which explains the partial but significant starch reduction observed under KNO_3 treatment.

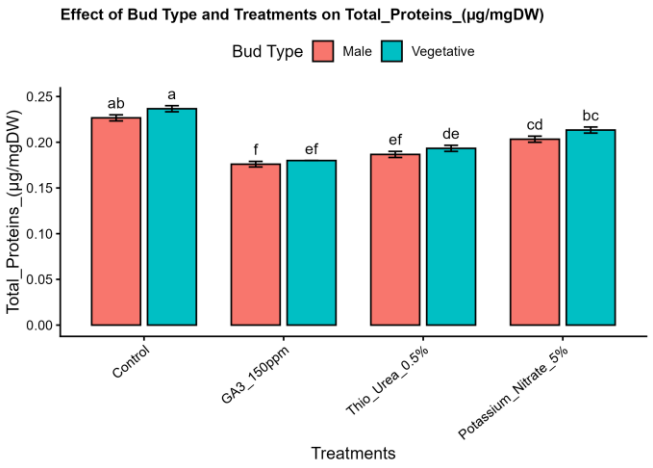

Fig. 2 : Effect of pre-flowering chemical treatments on total protein content (µg/mg DW) in male and vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Total protein content in bud tissue was significantly affected by pre-flowering chemical treatments (Figure 2; $F_{(3,16)}$ = 115.10, p < 0.001). In contrast to soluble sugars, all treatments reduced total protein relative to the untreated control. The control

recorded the highest protein concentrations in both male (0.227 µg/mg DW) and vegetative buds (0.237 µg/mg DW). GA_3 at 150 ppm produced the highest protein reduction, recording the lowest values in male (0.176 µg/mg DW) and vegetative buds (0.180 µg/mg DW). Thiourea (0.187 male; 0.193 vegetative) and KNO_3 (0.203 male; 0.213 vegetative) produced intermediate reductions. Vegetative buds retained marginally but significantly higher protein than male buds ($F_{(1,16)}$ = 12.37, p = 0.003). The bud type × treatment interaction was non-significant (p = 0.725).

The reduction in total protein content under dormancy-breaking treatments reflects protein catabolism during the transition from dormancy to active growth. During dormancy release, storage proteins are hydrolyzed to release free amino acids, which serve as nitrogen sources for the synthesis of new structural and enzymatic proteins required for cell proliferation and organ differentiation. This protein turnover is a well-documented feature of dormancy release in temperate fruit trees (El-Yazal & Rady, 2014). The highest protein reduction under GA_3 corresponds with its strongest dormancy-releasing effect, suggesting more extensive protein mobilization to support the accelerated metabolic activity observed in this treatment. The pattern is consistent with findings in 'Anna' apple trees, where dormancy-breaking substances including thiourea and KNO_3 reduced bud protein concentrations in conjunction with increased amino acid mobilization (El-Yazal & Rady, 2014).

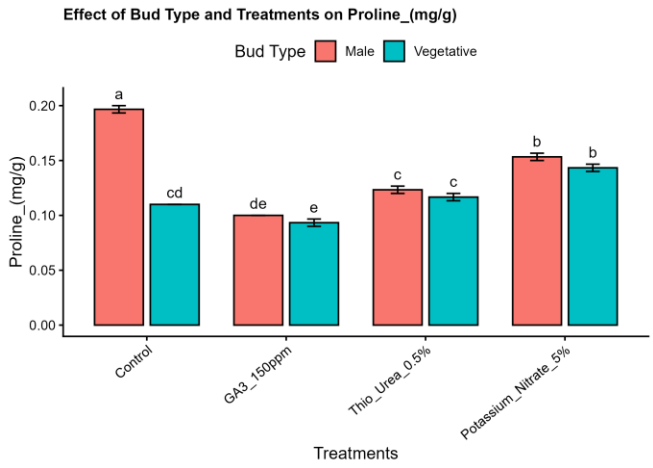

Fig. 3 : Effect of pre-flowering chemical treatments on proline content (mg/g) in male and Vegetative buds of walnut (*Juglans regia* L.) selection SKUAW-15.

Proline content was significantly influenced by both treatment and bud type, with a highly significant bud type × treatment interaction (Figure 3; $F_{(3,16)}$ = 93.50, p < 0.001), necessitating separate interpretation for each bud type. In male buds, the control recorded the highest proline concentration (0.197 mg/g), which was significantly greater than all treated plants. GA_3 produced the highest reduction in male bud proline (0.100 mg/g), followed by thiourea (0.123 mg/g) and KNO_3 (0.153 mg/g). In vegetative buds, the control recorded a substantially lower baseline proline concentration (0.110 mg/g) than in male buds, reflecting a lower dormancy-associated stress load. Only GA_3 reduced proline below the control level (0.093 mg/g), while thiourea (0.117 mg/g) and KNO_3 (0.143 mg/g) produced values marginally above the control, suggesting that the lower baseline proline in vegetative buds left limited scope for further reduction under these treatments. The high proline concentration in untreated male buds reflects the stress-associated proline accumulation that characterizes deep dormancy, where proline functions as an osmoprotectant and reactive oxygen species scavenger under the low-temperature, low-water-activity conditions of winter (Verbruggen & Hermans, 2008). The substantial reduction in proline under all treatments, and particularly under GA_3, indicates alleviation of dormancy-associated stress and transition to a metabolically active state where proline is no longer required at high concentrations for cellular protection. This study is supported by El-Yazal and Rady (2013), who reported that thiourea application elevated proline in apple buds during early dormancy release as a transient stress-response, while bud break percentage simultaneously improved suggesting that the timing of sampling relative to dormancy stage governs the direction of observed proline change. In the present study, buds were sampled 15 days after treatment application, at which point the actively treated buds had already progressed beyond peak dormancy stress, accounting for the lower proline observed in treated versus control buds. The lower baseline proline in vegetative buds compared to male buds in the control further suggests that vegetative buds experience less oxidative stress during dormancy, consistent with their delayed but less temperature-sensitive developmental trajectory.

Conclusion

The present investigation demonstrates that foliar application of GA_3 (150 ppm), thiourea (0.5%), and potassium nitrate (KNO_3, 5%) four weeks before expected flowering effectively accelerates dormancy release and advances flowering in walnut selection SKUAW-15 under Kashmir conditions. GA_3 at 150 ppm was consistently the most effective treatment across all phenological and biochemical parameters followed by Thiourea and KNO_3. Biochemically, the

treatments induced a coordinated shift in bud metabolism characterised by increased soluble sugar accumulation, reduced starch, reduced total protein, and reduced proline, collectively indicating more rapid and complete dormancy release. The magnitude of these biochemical changes was proportional to the dormancy-breaking efficacy of each treatment, with GA_3 producing the highest starch-to-sugar conversion and the most pronounced protein mobilisation. Critically, however, all three treatments advanced male and female flowering by an equal margin, maintaining the inherent 3-day dichogamous offset across all treatments. The consistent maintenance of the dichogamy offset across all treatments indicates that these chemicals act uniformly on shared dormancy pathways, providing a baseline reference for future studies targeting type-specific flowering manipulation in walnut.

Acknowledgement

The authors thank Division of Fruit Science, FOH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India for supporting this study.

References

Alebidi, A., Al-Obeed, R., Al-Saif, A., Soliman, S., Al-Mehmadi, R., Elsalam, I. N. A., Aziz, A. and Osaili, T. M. (2023). Effect of potassium nitrate on the growth, flowering, and biochemical attributes of date palm. *Agronomy*, **13(2)**, 487.

Bohnert, H. J. and Sheveleva, E. (1998). Plant stress adaptations, Making metabolism move. *Current Opinion in Plant Biology*, **1(3)**, 267–274.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72(1–2)**, 248–254.

Campoy, J. A., Darbyshire, R., Dirlewanger, E., Garcia, J. Q. and Wenden, B. (2018). Yield potential definition of the chilling requirement reveals likely underestimation of the risk of climate change on winter chill accumulation. *International Journal of Biometeorology* **63(2),** 183-192.

Charrier, G., Poirier, M., Bonhomme, M., Lacointe, A. and Améglio, T. (2013). Frost hardiness in walnut trees (*Juglans regia* L.), How to link physiology and modelling? *Tree Physiology*, **33(11),** 1229–1241.

Chen, H., Romo-Leroux, P. A. and Salin, M. L. (2006). Oxygen-evolving photosynthesis and catalase activity in catalase-deficient tobacco. *Free Radical Biology and Medicine*, **40(3),** 380–387.

Chmielewski, F. M. and Götz, K. P. (2017). Identification of chilling and heat requirements of cherry trees under controlled conditions. *Journal of Horticultural Science & Biotechnology*, **92(5)**, 470–478.

Elsabagh, A. S. (2014). Influences of potassium nitrate, gibberellin and benzyl adenine on bud break, fruit set and branch induction of almond trees. *Acta Horticulturae*, **1028(2),** 359–366.

El-Yazal, M. A. S. and Rady, M. M. (2013). Foliar-applied Dormex™ or thiourea-enhanced proline and biogenic amine contents and hastened breaking bud dormancy in 'Ain Shemer' apple trees. *Trees*, **27(1),** 161–169.

El-Yazal, M. A. S. and Rady, M. M. (2014). Exogenous dormancy-breaking substances positively change endogenous phytohormones and amino acids during dormancy release in 'Anna' apple trees. *Plant Growth Regulation*, **74(2)**, 197–207.

Fadón, E., Rodrigo, J., Arévalo, M. A. and Herrero, M. (2015). Flower bud development in sweet cherry (*Prunus avium* L.) viewed inside out. *International Journal of Molecular Sciences*, **16(3)**, 5488–5503.

Fayek, M. A., Fayed, T. A., El-Said, E. H. and El-Hamed, E. E. A. (2008). Utilization of some chemicals for synchronizing time of male and female flowers in pecan (*Carya illinoinensis* Koch). *Research Journal of Agriculture and Biological Sciences*, **4(6),** 813–818.

Fisher, M. (1950). Colour atlas of fruit cultivar (p. 320).

Hassankhah, A., Rahemi, M., Mozafari, M. R. and Vahdati, K. (2018). Flower development in walnut, Altering the flowering pattern by gibberellic acid application. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **46(2),** 700–706.

Ionescu, I. A., Møller, B. L. and Sánchez-Pérez, R. (2017). Chemical control of flowering time. *Journal of Experimental Botany*, **68(3),** 369–382.

Irigoyen, J. J., Emérich, D. W. and Sánchez-Díaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum*, **84(1),** 55–60.

Mansouri, H., Asrar, Z. and Szopa, J. (2011). Changes in essential oil compounds of *Artemisia annua* during cold pretreatment and dormancy breaking. *Journal of Plant Physiology*, **168(11),** 1213–1219.

Meier, U. (Ed.). (2001). *Growth stages of mono- and dicotyledonous plants, BBCH Monograph* (2nd ed.). Federal Biological Research Centre for Agriculture and Forestry.

Mitra, S. K. and Sanyal, D. (2000). Effect of cincturing and chemicals on flowering of litchi. *Acta Horticulturae*, **558(3),** 243–246.

Pollock, C. J. and Lloyd, E. J. (1987). The effect of low temperature upon starch, sucrose and fructan synthesis in leaves. *Annals of Botany*, **60(2),** 231–235.

Protacio, C. M. (2000). A model for potassium nitrate-induced flowering in mango. *Acta Horticulturae*, **509(1),** 503–510.

Qu, Y., Chen, X., Mao, X., Huang, P. and Fu, X. (2022). Transcriptome analysis reveals the role of GA3 in regulating the asynchronism of floral bud differentiation and development in heterodichogamous *Cyclocarya paliurus* (Batal.). *International Journal of Molecular Sciences*, **23(12),** 6763.

Rose, R., Rose, C. L., Omi, S. K., Forry, K. R., Durall, D. M. and Bigg, W. L. (1991). Starch determination by perchloric acid vs enzymatic digestion, Comparison using zelkova plants. *Tree Physiology*, **8(3),** 295–297.

Sritontip, C., Khaosumain, Y., Changjaraja, S. and Poruksa, R. (2005). Effect of potassium chlorate, potassium nitrate, sodium hypochlorite and thiourea on off-season flowering and photosynthesis of 'Do' longan. *Acta Horticulturae*, **665(2),** 301–307.

Verbruggen, N. and Hermans, C. (2008). Proline accumulation in plants, A review. *Amino Acids*, **35(4),** 753–759.

Wahid, A., Basra, S. M. A. and Farooq, M. (2017). Thiourea, A molecule with immense biological significance for plants. *International Journal of Agriculture and Biology*, **19(4),** 911–920.

Wolak, R. J. and Couvillon, G. A. (1990). Time of thiourea-KNO_3 application on the rest requirement and bud development in 'Loring' peach. *HortScience*, **25(9),** 1149.