

Application of Phosphorus and Determination of Heat Units to Increase Productivity and Isoflavone Content of Several Varieties of Edamame Soybeans

Nadya Inri Meiana Manullang^A, Maya Melati^{*B}, Munif Ghulamahdi^B

^A Agronomy and Horticulture Study Program, Graduate School of IPB University (Bogor Agricultural University), Bogor 16680, Indonesia

^B Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor 16680, Indonesia

*Corresponding author; email: maya_melati@apps.ipb.ac.id

Abstract

Edamame is rich in isoflavones, which offer notable health and beauty benefits. Phosphorus influences the biosynthesis of isoflavones in edamame, making this nutrient an important factor in crop management. Edamame soybeans are harvested at the R6 stage, when pods are fully developed yet still green, a timing that is critical for ensuring optimal quality. Harvest maturity is determined using accumulated heat units, a method that calculates the temperature differences at the planting site to assess crop readiness accurately. This study evaluated the effects of phosphorus fertilization and variety on edamame productivity and isoflavone content. The experiment was conducted at the IPB Experimental Station in Bogor, Indonesia (coordinates: -6.548927° S, 106.734462° E), using a randomized complete block design with three replications. The first factor was phosphorus fertilizer applied at rates of 0, 18, 36, and 54 kg P₂O₅ per hectare. In contrast, the second factor was edamame variety ("Biomax 1", "Biomax 2", and "Detam 1" as a check variety for isoflavone content). The results showed no significant interaction between phosphorus fertilization and variety for several growth parameters, including leaf phosphorus content, leaf number, branch number, relative growth rate, and pod weight per plant. However, significant differences among varieties were observed for leaf number and seed weight per plant. Phosphorus fertilization did not significantly affect branch number or pod weight, but it did influence isoflavone content, with different phosphorus doses increasing genistein accumulation in certain varieties. The study also highlighted the impact of temperature on plant growth, as evidenced by significant differences in accumulated crop heat units (CHU) among the varieties: "Biomax 1" required 2029.50–2050.75°C days, "Biomax 2" required 2070.20–2132.25°C days, and "Detam 1" required 2218.85–2256.05°C days. The highest

isoflavone content was recorded in "Biomax 2" with the application of 36 kg P₂O₅ per hectare.

Overall, these findings underscore the complex relationship between soil conditions, nutrient availability, and varietal selection in determining edamame yield and quality. This research provides valuable insights for optimizing edamame production and enhancing its nutritional value.

Keywords: growth degree days, leaf nutrient content, net assimilation rate, number of pods, secondary metabolites

Introduction

Edamame soybeans (*Glycine max* L.) are a popular vegetable in Asia, widely enjoyed both as a snack and as an ingredient in various dishes (Mozzoni and Chen, 2019). Indonesia currently supplies about 3% of the global demand for edamame, with the majority sourced from China and Taiwan; productivity in Indonesia reaches approximately 8.8 tons per hectare (Saputra and Anggraini, 2021). Edamame contains phytochemical components such as isoflavones, sterols, and saponins (Xu et al., 2016). Edamame soybeans are generally harvested when the seeds are still young or at the R6 phase when the pods are 80-90% full (Tsindi et al., 2019). The edamame seeds are large and contain high amounts of protein, fat, calcium, vitamin E, and isoflavones (Zeipina et al., 2022). Due to its excellent nutritional profile and associated health benefits-including supporting heart health, bone health, and cholesterol management, the demand for edamame soybeans continues to rise. Isoflavones are synthesized via the phenylpropanoid pathway from which plants produce most of the secondary metabolites (Ningsih et al., 2018; Deorukhkar and Ananthanarayan, 2021). The

isoflavone content in edamame soybeans includes several components, such as genistein, daidzein, and glycitein (Akitha Devi et al., 2018). Edamame soybean isoflavones have a chemical structure similar to the hormone estrogen, making them beneficial in the health field to prevent certain diseases (Lee et al., 2021), such as breast cancer, ovarian cancer, menopausal symptoms, cardiovascular diseases, and osteoporosis (Parker et al., 2020). In beauty, it is used to prevent aging and dry skin (Sohn et al., 2021). Several studies have shown that isoflavones have antioxidant, phytoestrogenic, and anti-inflammatory properties.

The promising potential of edamame cultivation requires optimal cultivation techniques. Several factors, including the type, dosage, method, and timing of application, influence proper fertilization application (Hutabarat et al., 2022). Phosphorus is an immobile macronutrient in plants and unavailable in organic or inorganic forms. (Lazali and Drevon, 2021), which can lead to plant deficiencies. Phosphorus also plays a role in photosynthesis and is a component of ATP (Bagale, 2021). The isoflavone content in edamame soybeans can also be determined by the variety used, and using superior varieties can increase both productivity and nutritional content in plants. Soybean varieties can affect plant phases, such as prolonging the vegetative phase, which in turn affects flowering and pod maturity (Moinuddin et al., 2023). The effects of fertilization and variety on increasing isoflavone content need further study to determine the proper fertilization dosage and the varieties that produce high isoflavone content.

The heat unit accumulation method is reliable for determining the maturity stage of fruits or vegetables by considering temperature and light intensity variations across growing locations and seasons. This method calculates the actual average temperature by factoring in the plant's base temperature until it reaches optimal harvest maturity (Abdurrohman et al., 2018). Environmental factors significantly affect plant conditions, damage levels, and the quality of harvested fruits or vegetables. These factors also influence plant productivity during specific seasons and their adaptability to varying conditions (Subrahmaniyan et al., 2021). Temperature plays a critical role in photosynthesis and the translocation of photosynthates to seeds, thereby reducing potential losses of photosynthates. However, the relationship between temperature and the plant's assimilation rate may lead to inaccuracies when extreme temperature variations occur under diverse environmental conditions (Zhou and Wang, 2018). No studies have investigated the simultaneous interaction between soybean varieties, phosphorus fertilizer dosages,

productivity, and isoflavone content. This study aims to assess the growth and productivity of soybean and the isoflavone compounds produced by edamame under varying phosphorus dosages and three soybean varieties. Additionally, it seeks to examine the interactive effects of phosphorus fertilizer dosages and soybean varieties on both productivity and isoflavone content and to determine the heat units required for harvesting three edamame varieties under different phosphorus dosages.

Materials and Methods

The experiment was conducted from March to July 2024 at the IPB Experimental Station in Cikarawang, Darmaga, Bogor Indonesia. The materials used in the experiment were Edamame soybean seeds of the "Biomax 1" and "Biomax 2" varieties, black soybean seeds of the "Detam 2" 1 variety, dolomite, chicken manure, urea, SP-36, and KCl fertilizers as sources of N, P, and K, respectively.

Experimental Design and Procedures

The experiment used a factorial randomized complete block design with two factors and three replications. The first factor was phosphorus fertilizer dosage, consisting of four levels: 0, 18, 36, and 54 kg.ha⁻¹ P₂O₅. The second factor was soybean varieties, consisting of "Biomax 1" and "Biomax 2" (edamame), and the black soybean variety "Detam 1", a comparison of its isoflavone content.

The plot size of each experimental unit was 1.25 m × 4 m, and the planting distance was 25 cm × 20 cm. The pod yield was calculated from a size 1.2 m² production. The basal fertilizer applied was chicken manure at a rate of 5 ton.ha⁻¹ and dolomite at 2 ton.ha⁻¹, applied two weeks before planting. The nitrogen (N) fertilizer at a rate of 50 kg of urea per ha, (K) fertilizer at 75 kg K₂O per ha, and phosphorus (P) fertilizer, with dosages according to the treatments: 0, 18, 36, and 54 kg.ha⁻¹ P₂O₅, were applied on the planting date.

The variables to measure included the physiological response and production of edamame soybeans, which were: 1) the nutrient content of the soil before planting, 2) nitrogen, phosphorus, and potassium content in plant tissues, 3) plant morphology (leaf and branch numbers), 4) relative growth rate and net assimilation rate, 5) production of edamame and its components (pod number, pod weight per plant and 100 seed weight), 6) isoflavone content of edamame seed, and 7) the heat unit of edamame soybean plants.

Soil Analysis

The nutrient analysis was performed using soil samples collected through composite sampling. The composite soil samples were taken randomly from five points in the field to be planted. The soil total N was analyzed using the Kjeldahl method, while P and K were analyzed using spectrophotometry and atomic absorption spectroscopy (AAS).

Leaf Nutrient Analysis

Leaf nutrient analysis was conducted six weeks after planting (at maximum vegetative stage). The mature leaves were sampled, air-dried, and then oven-dried at 60°C until they reached a constant weight. The dry leaf tissue samples were then analyzed for N, P, and K content using the wet ashing method, which involved the use of 65% HNO₃ and 60% HClO₄, followed by measurement with spectrophotometry.

Net Assimilation Rate and Relative Growth Rate

Weekly Relative Growth Rate (RGR) was calculated based on the dry weight of the plant per unit time. RGR was determined 4-6 weeks after planting (WAP). The formula used is:

$$\text{RGR (g.g}^{-1} \text{ per week)} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Description:

W1 and W2 = Dry weight of the plant at the first and second observations

T1 and T2 = Plant age at the first and second observations.

Weekly Net Assimilation Rate (NAR) was determined from the plant's dry weight per unit leaf area over a specific period. NAR was calculated from the period at 4-6 Weeks After Planting (WAP). The formula used is:

$$\text{NAR (g.cm}^{-2} \text{ per week)} = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where

W1 and W2 = Dry weight of the plant at the first and second observations

T1 and T2 = Plant age at the first and second observations

A1 and A2 = Leaf area of the plant at the first and second observations

Isoflavone Content Analysis

The reverse-phase HPLC method is a validated and simple approach for quantifying free isoflavone aglycones (daidzein and genistein). Isoflavone analysis in edamame was conducted at the Tropical

Biopharmaca Research Center (Trop BRC), IPB University, Bogor, Indonesia. The maceration process was done twice, and all samples were analyzed using HPLC (high-performance liquid chromatography). The method used an LC-20 AD, Photo Diode-Array (PDA) detector (Shimadzu, Japan), and the column used was C-18 (150 mm x 4.6 mm, 5 μm). The mobile phase used water containing 0.1% glacial acetic acid and methanol (v/v) with a ratio of 53:47, a flow rate of 1.0 mL per min, and a 20 μL injection volume.

Crop Heat Unit Measurements

The crop heat unit measurement was carried out by collecting temperature data from planting to harvest using a data logger installed in the field. The equipment used was the Elitech GSP6 Thermometer and Hygrometer Data Logger. The collected data were then calculated to obtain the heat unit values.

$$\text{Crop heat unit (}^{\circ}\text{C days)} = \frac{(T_{\max} + T_{\min})}{2} - T_{\text{base}}$$

where

Tmax : the highest daily temperature (°C)

Tmin : the lowest daily temperature (°C)

Tbase : the base temperature (°C), which is 10°

Results and Discussion

Soil Analysis

The soil analysis before the experiment showed that the soil pH was low and slightly acidic. The organic carbon content was medium, while the nitrogen content was low. The available phosphorus and potassium in the soil were high (Table 1).

Leaf Nutrient Analysis

The results showed no significant effect of the interaction between phosphorus fertilizer application and variety on leaf phosphorus content. The soybean varieties had different leaf nitrogen content; the highest N concentration was found in "Detam 1" (Table 2). An interaction was observed between phosphorus and variety in leaf potassium content. Leaf K of "Biomax 2" was higher with the highest P dose, while in "Detam 1," leaf K was lower with increasing P doses (Table 3).

Relative Growth Rate

The results showed that the interaction effect between phosphorus application and variety and each single treatment factor did not affect the relative growth rate of the plant (Table 4). During the initial growth phase, plants absorb nutrients and water to form

Table 1. Soil chemical properties before planting edamame soybean

Soil parameter	Value	Status
pH H ₂ O	5.70	Slight acidic
pH KCl	5.04	Acidic
C-Organic (%)	1.33	Medium
Total N (%)	0.20	Low
Available P (P ₂ O ₅ , ppm)	36.78	High
K (cmol(+)/kg)	46.41	Very High
CEC (cmol(+)/kg)	14.84	Low

Note: Soil chemical properties criteria according to Indonesian Soil Research Institute (2005).

Table 2. Effect of different soybean varieties and phosphorus fertilizer dosages on leaf nitrogen and phosphorus

Treatments	Leaf nutrient content (%)	
	Nitrogen	Phosphorus
Edamame soybean varieties		
“Biomax 1”	4.55 ab	0.31
“Biomax 2”	4.42 b	0.31
“Detam 1”	4.70 a	0.31
P fertilizer rates (kg P ₂ O ₅ per ha)		
0	4.63	0.32
18	4.58	0.30
36	4.47	0.31
54	4.53	0.32

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%.

Table 3. The interaction effects of different soybean varieties and phosphorus fertilizer dosages on leaf potassium content

P fertilizer rates (kg P ₂ O ₅ per ha)	Edamame soybean varieties		
	“Biomax 1”	“Biomax 2”	“Detam 1”
0	1.79 a	1.67 b	2.38 a
18	1.05 b	1.64 b	1.93 a
36	1.45 b	1.63 b	2.24 a
54	1.84 a	1.95 a	1.29 b

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%.

Table 4. Effects of different soybean varieties and phosphorus fertilizer dosages on relative growth rate

Treatments	Relative growth rates (g.g. per week)
Variety	
“Biomax 1”	1.01
“Biomax 2”	1.16
“Detam 1”	1.06
P fertilizer rates (kg P ₂ O ₅ per ha)	
0	1.03
18	1.05
36	1.10
54	1.12

chlorophyll in the leaves as part of photosynthesis. The photosynthates produced are then directed toward supporting vegetative growth. The availability of nutrients plays a crucial role in facilitating photosynthesis and tissue formation (Simatupang et al., 2023)

Leaf Number and Branch Number

Our study found that the leaf number increased with plant age until the end of the vegetative phase. Phosphorus fertilization did not influence leaf number, but the edamame variety significantly affected leaf number in 6 WAP, with “Detam 1” producing the highest leaf number (Table 5). The leaf number is influenced by genotype, plant height, the number of nodes, and the number of branches (Harahap et al., 2024).

Edamame varieties vary significantly in the number of productive branches, with “Detam 1” having the highest number of productive branches (Table 5). The number of branches in a plant can be influenced by its genetics (Xu et al., 2021). Phosphorus application did not affect branch proliferation, as phosphorus is primarily involved in plant root and seed formation.

Phosphate can promote root development, as well as pod and seed maturation (Adnan et al., 2021).

Net Assimilation Rates

The net assimilation rate is an indicator of a plant's efficiency in converting sunlight into biomass, considering the amount of biomass lost through respiration (Kiri et al., 2023). Based on the current study, the net assimilation rate was affected by the interaction between phosphorus fertilization and variety (Table 6). The NAR of “Biomax 1” decreased with higher P rates, while the NAR of “Biomax 2” and “Detam 1” were higher with the highest rate of P fertilizer. The higher NAR in these two varieties may relate to the increasing leaf number with higher P rates (Table 5). The net assimilation rate increases in line with the rise in leaf area ratio up to a certain point, after which it begins to decline. In canopies with a high leaf area ratio, younger leaves absorb the light, exhibit higher photosynthesis rates, and translocate a significant portion of the photosynthates to other parts of the plant, including the lower leaves. In contrast, photosynthesis in the lower leaves slows down due to shading by the upper leaves (Mahmudi et al., 2022).

Table 5. Effect of different soybean varieties and phosphorus fertilizer dosages on leaf number and branch number at 4 to 6 weeks after planting (WAP)

Treatment	Leaf number		Branch number
	4 WAP	6 WAP	
Variety			
“Biomax 1”	16.1	31.2 b	9.5 b
“Biomax 2”	16.3	24.9 c	10.0 b
“Detam 1”	17.1	44.0 a	21.4 a
P fertilizer rates (kg P₂O₅ per ha)			
0	17.2	30.4	13.6
18	16.2	34.0	13.0
36	16.5	34.0	13.0
54	16.1	35.4	15.0

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%.

Table 6. Interaction effect of different soybean varieties and phosphorus fertilizer dosages on the net assimilation rate of plants

P fertilizer rates (kg P ₂ O ₅ per ha)	Net assimilation rates (g.cm ² per week)		
	“Biomax 1”	“Biomax 2”	“Detam 1”
0	0.043 a	0.016 b	0.027 a
18	0.053 a	0.027 b	0.037 a
36	0.026 b	0.041 a	0.047 a
54	0.019 b	0.019 b	0.016 b

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%; Data were transformed using the square root ($\sqrt{x + 0.5}$).

Pod Number, Pod Weight per Plant, 100-Seed Weight

This study showed no interaction effect between phosphorus fertilization and variety on pod weight per plant. Seed weight per plant varies with edamame varieties, but phosphorus rates did not affect the yield components (Table 7). Different soybean varieties yield different production levels and seed sizes, indicating that variety significantly impacts soybean growth and yield (Amin et al., 2021). Although the pod number of “Biomax 2” was lower than others, this variety had the highest pod weight per plant. This indicated that the single pod weight of “Biomax 2” was higher than the other varieties.

The impact of P fertilizer was not in line with previous research showing that phosphorus fertilization significantly increased the number of pods per plant (Turuko and Mohammed, 2014). High P available in the soil (Table 1) might be sufficient for the plant. The leaves’ N, P, and K concentrations (Tables 2 and 3) are already within the adequate range. An increase in phosphorus (P) can inhibit nitrogen (N) and potassium (K) uptake, as indicated by the negative mean values in the P: N and P: K ratios. In other words, excessive P application may hinder the plant’s ability to absorb nitrogen and potassium efficiently. The standard deviation values reflect the variation in this data. For the P/N ratio, the variation is relatively small (± 0.09), while for the P/K ratio, it is slightly higher (± 0.15), indicating that the effect of phosphorus on potassium uptake is more variable compared to its impact on nitrogen (Ferreira et al., 2024).

Weight of Filled Pods and Number of Filled Pods Per Plot

The number of pods per plot was not influenced by the interaction between phosphorus fertilizer dose

and variety or by P fertilizer rates only. However, the number of pods per plot was different among varieties, with the highest number observed in the “Detam 1” variety (Table 8). The highest weight of filled pods per plot was found in the “Biomax 1” variety. Different from Table 7, pod weight and pod number per plot of “Biomax 2” were the lowest. In “Biomax 2” many plants did not grow, which resulted in a lower yield. The fulfillment of nutrient requirements in plants enables optimal metabolic processes, leading to the accumulation of metabolic products that contribute to seed formation, ultimately resulting in maximum seed size and weight (Kurniawati et al., 2022). Factors such as seed filling, harvesting, and handling influence seed quality, while temperature and moisture content trigger chemical and metabolic changes in the seeds (Ali et al., 2019).

Isoflavone Content

The results revealed a notable interaction between phosphorus application rates and plant variety. “Biomax 1” exhibited the highest isoflavone content when fertilized with 18 kg P₂O₅, reaching 197.6 µg.g⁻¹. “Biomax 2” demonstrated its maximum isoflavone content at a phosphorus dose of 36 kg P₂O₅, with a value of 370.5 µg.g⁻¹. “Detam 1”, on the other hand, showed the highest isoflavone content at 18 kg P₂O₅, reaching 445.1 µg.g⁻¹. Isoflavones are plant-specific compounds predominantly found in legumes. Phosphorus fertilization has been shown to significantly enhance the accumulation of genistein compared to other isoflavones, such as daidzein and glycitein, as indicated by total aglycone production (Wang et al., 2019) (Table 9).

Table 7. Effect of different soybean varieties and phosphorus fertilizer dosages on pod number, pod weight per plant, and 100 seed fresh weight

Treatment	Pod number	Pod weight per plant (g)	100-seed fresh weight (g)
Variety			
“Biomax 1”	17.1 a	36.28 b	54.75 a
“Biomax 2”	14.1 b	41.93 a	52.12 a
“Detam 1”	17.4 a	34.58 b	20.61 b
P fertilizer rates (kg P₂O₅ per ha)			
0	15.0	37.69	42.21
18	17.3	35.79	40.54
36	16.8	37.76	45.90
54	15.8	38.17	41.32

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%; Data were transformed using the square root ($\sqrt{x + 0.5}$).

Table 8. Effect of different soybean varieties and phosphorus fertilizer dosages on the weight of filled pods per plot and the number of filled pods per plot

Treatments	Weight of filled pods per plot (g.1.2 m ⁻²)	Number of filled pods per plot
Variety		
“Biomax 1”	902.50 a	299.3 a
“Biomax 2”	436.00 b	193.8 b
“Detam 1”	483.58 b	294.5 a
P fertilizer rates (kg P₂O₅ per ha)		
0	571.22	220.4
18	600.22	308.3
36	655.56	282.2
54	602.44	239.1

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%; Data were transformed using the square root ($\sqrt{x + 0.5}$).

Table 9. Edamame seed isoflavone content of different soybean varieties at various dosages of phosphorus fertilizer

P fertilizer rates (kg P ₂ O ₅ per ha)	Isoflavone content (µg.g ⁻¹ dry weight)		
	“Biomax 1”	“Biomax 2”	“Detam 1”
0	147.8 b	348.9 b	198.1 b
18	197.6 a	347.2 b	445.1 a
36	194.1 a	370.5 a	298.0 a
54	173.4 b	199.5 c	218.9 b

Notes: values followed by the same letter in the same column are not significantly different at DMRT α 5%.

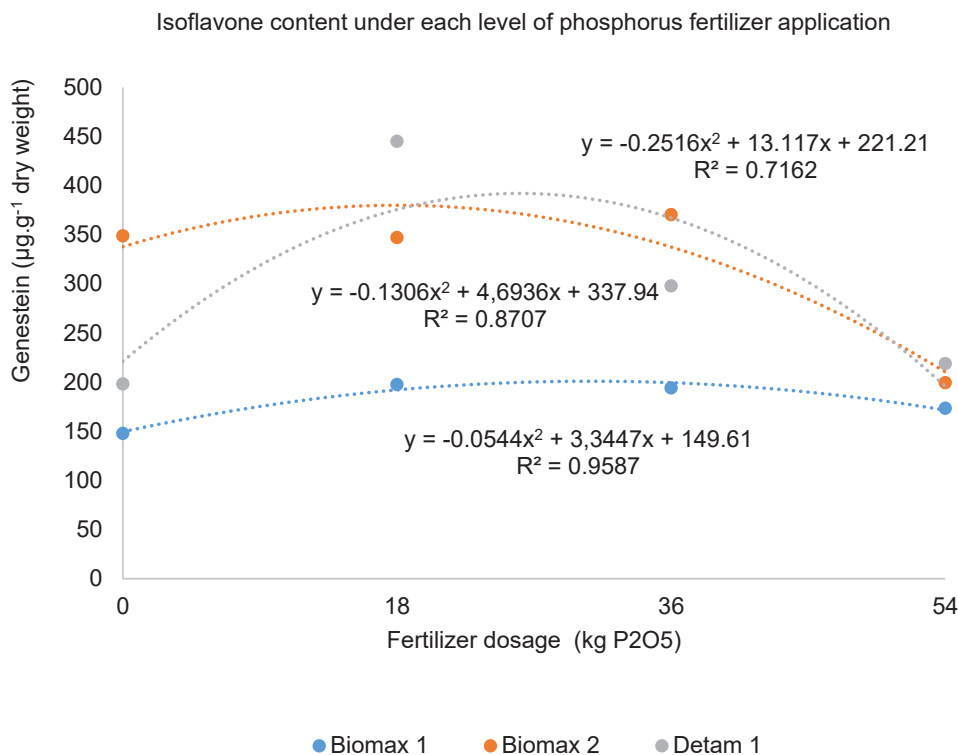


Figure 1. Regression of isoflavone content for each variety in response to phosphorus fertilizer dose

Regression of Fertilizer Dosage and Genistein Content in Each Edamame Soybean Variety

The regression test shows that the isoflavone genistein content in the “Biomax 1” variety increases with the increasing dosage of P₂O₅ fertilizer up to an optimal point, after which it slightly decreases. The quadratic equation with R² = 0.9587 indicates a strong relationship between fertilizer dosage and isoflavone content. The highest isoflavone content is achieved at a fertilizer dosage of approximately 36 kg P₂O₅. In the “Biomax 2” variety, the isoflavone genistein content follows a similar pattern, increasing to an optimal dosage and then decreasing afterward. The quadratic equation with R² = 0.8707 indicates a strong correlation between fertilizer dosage and isoflavone content. The highest isoflavone content is achieved at a dosage of around 36 kg P₂O₅. The “Detam 2” variety exhibits an increasing trend in isoflavone content with rising fertilizer dosage, reaching an optimal range around 18-36 kg P₂O₅ before declining at 54 kg P₂O₅. The R² value of 0.7162 indicates a relatively strong relationship, although not as strong as in “Biomax 1” and “Biomax 2”. The highest isoflavone content is obtained at a fertilizer dosage of approximately 36 kg P₂O₅.

Crop Heat Unit

The accumulation of crop heat units (CHU) from planting to harvest for the “Biomax 1” variety ranged from 2029.50 to 2050.75°C days, while the “Biomax 2” variety had a total CHU of 2070.20 to 2132.25°C days. The “Detam 2” variety exhibited a CHU range of 2218.85 to 2256.05°C days. Based on the total CHU results, “Biomax 1” had a lower total CHU than the “Detam 1” variety. This variation is attributed to differences in variety and phosphorus fertilizer dose, leading to variations in the total CHU obtained. Temperature significantly influences plant growth, from the emergence of the first leaf to seed production (Table 10). The duration of each growth stage determines the amount and distribution of nutrients throughout the plant. Additionally, temperature affects the plant’s ability to adapt to its environment (Subrahmaniyan et al., 2021).

The crop heat unit represents the total accumulated temperature required for plant growth and development. The current study provides information on the CHU of edamame soybeans under the conditions of environmental sites. (Parthasarathi, 2013) such as from seeding to the harvest stage.

Table 10. Total crop heat unit of edamame soybeans from planting to dry harvest with different varieties and phosphorus fertilizer application

Biomax 1				
Growth phase	Rates of P fertilizer (kg P ₂ O ₅)			
	0	18	36	54
Early planting–Early generative (VE-R1)	462.90	444.30	444.30	444.30
Early generative–Young pod harvest (R1-R6)	807.60	788.00	788.00	788.0
Young pod harvest–Dry seed harvest (R6-R8)	726.20	705.65	705.65	705.65
Total crop heat unit (°C days)	1996.70	1937.95	1937.95	1937.95
Biomax 2				
Growth phase	Rates of P fertilizer (kg P ₂ O ₅)			
	0	18	36	54
Early planting–Early generative (VE-R1)	440.70	440.70	440.70	440.70
Early generative–Young pod harvest (R1-R6)	960.85	940.25	919.80	919.80
Young pod harvest–Dry seed harvest (R6-R8)	606.50	585.50	585.50	585.50
Total crop heat unit (°C days)	2008.50	1966.45	1946.00	1946.00
Detam				
Growth phase	Rates of P fertilizer (kg P ₂ O ₅)			
	0	18	36	54
Early planting–Early generative (VE-R1)	423.25	423.25	406.95	406.95
Early generative–Young pod harvest (R1-R6)	993.75	993.75	974.35	952.65
Young pod harvest–Dry seed harvest (R6-R8)	645.70	624.80	624.80	624.80
Total crop heat unit (°C days)	2062.70	2041.80	2006.10	1984.40

Temperature is a key factor for the timing of biological processes, and hence the growth and development of plants. CHU can be used as an estimate to determine the harvest age of plants in different locations according to each area's base temperature, maximum temperature, and minimum temperature. CHU index is considered one of the best methods for assessing the impact of temperature on plant growth. Research has shown a strong correlation ($R^2 = 0.74$) between CHU and the average soybean yield (Wen et al., 2022). In warmer regions, the growth process occurs more rapidly; conversely, in cooler climates, CHU increases, extending the plant's growth cycle. In addition to temperature, rainfall and water availability significantly affect CHU efficiency, particularly in nutrient absorption. In high-rainfall areas, nutrient leaching may reduce phosphorus fertilizer effectiveness. Meanwhile, in drier regions, limited water availability can hinder nutrient uptake, slowing plant growth and delaying the attainment of optimal CHU. Exposure to high temperatures during the grain-filling phase, including increased nighttime temperatures, can affect plant growth. The rise in minimum temperatures during the reproductive stage harms yield. Warmer temperatures during the vegetative phase tend to have a more minor negative impact since they remain within the optimal temperature range for the plant (Hatfield and Prueger, 2015). The duration of the generative phase also influences CHU requirements. "Biomax 2" and "Detam 2" have a longer early generative–young harvest phase than "Biomax 1". These varieties may struggle to obtain sufficient CHU for optimal growth if grown in regions with shorter growing seasons. Therefore, adjusting fertilizer applications and selecting the appropriate variety based on environmental conditions is essential to ensure plants achieve optimal CHU and maximize yield.

Conclusions

Edamame variety "Biomax 1" treated with 18 kg P_2O_5 per hectare produced the highest number of leaves, branches, net assimilation rate (NAR), pods, and 100-seed weight. This variety also recorded the greatest pod weight per plot and the highest number of filled pods per plot. In contrast, "Biomax 2" treated with 36 kg P_2O_5 per hectare yielded the highest isoflavone content. "Biomax 1" accumulated fewer total crop heat units, indicating a shorter growing period than the other varieties. The study further demonstrated that each edamame variety has distinct thermal requirements, as shown by differences in accumulated crop heat units: "Biomax 1" required 2029.50–2050.75°C days, "Biomax 2" needed 2070.20–2132.25°C days, and "Detam 2"

required 2218.85–2256.05°C days. Understanding these varietal differences can help optimize edamame production and enhance nutritional quality. The findings provide a foundation for further research and broader application in edamame cultivation.

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