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Effects of Gibberellic Acid (GA3) Application on The Plant Growth and Seed Production of Pinto Peanut (*Arachis pintoi* Krap & Greg)

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Abstract

Pinto peanut (*Arachis pintoi* Krap & Greg.) is a legume usually used as a cover crop, bio mulch in fruit and vegetable plantations, ornamental plants, and animal feed. Pinto peanut has many benefits; through symbiosis with rhizobacteria, they can fix nitrogen, as ground cover can reduce the risk of landslides, inhibit weed growths, and is a source of nectar for bees. *Arahcnis pintoi* can be propagated vegetatively or generatively, but generative propagation is hard to conduct in the tropics because it takes a long time for the plants to produce seeds. Our study was conducted to determine the effect of GA_3 application on the seed production of *A. pintoi*. The experiment was arranged in a single-factor randomized complete block design with GA_3 concentrations of 0, 75, 150, 225, and 300 ppm. An orthogonal polynomial test was conducted to determine the effective concentrations for GA_3 on seed formation and viability. Harvested seeds were stored for one month, then sown on sand medium; the seeds were soaked in 1% KNO_3 solution to break seed dormancy prior. Our study showed that the GA_3 effective concentration range from 130.69 ppm to 137.16 ppm, indicated by the increase in the number of flowers at 105, 120, 135, and 150 DAT up to 28.59% compared to control. GA_3 at 126.80 ppm can also increase the number of harvested pods by 18.16%. The effect of GA_3 on vegetative growth was shown by the increase in the growth of new individual plants concentration, i.e. 53.25 new individual plants with GA_3 application of 141.88 ppm.

Keywords: dormancy, gibberellin acid, growth regulators, KNO_3, legume cover crop.

Introduction

Pinto peanut (*Arachis pintoi*) is a low-growing perennial species that spreads by stolons or runners. The leaf of pinto peanut has four leaflets in each leaf, usually round-tipped and light green; the flowers are predominantly yellow. The seed production occurs in pods; at the base of a flower, a peg grows to a length of 5 to 30 cm, and penetrates the soil surface where it will produce a pod (Sanchez et al., 2020). Pintoi peanut pods usually contain one seed; however, two or three seeds may be observed (Sanchez et al., 2020). One of the main characteristics of pintoi peanut pods is that they drop from the pegs after maturity; therefore it requires soil separation from the pods to obtain the seeds.

Arachnis pintoi grows by forming a strong weave with roots or tendrils that will grow when the stem is in direct contact with the soil. In the tropics and sub-tropics, pinto peanuts can grow in the lowlands and highlands under 70–80% shade. In Indonesia, *Arachnis pintoi* is popularly known as an ornamental peanut (Balittan, 2004).

Arachnis pintoi has a fast growth rate during the vegetative phase and a slow growth rate during the generative phase; they bloom throughout the year with 40–65 flowers per m^2 per day (Sumiahadi, 2014). After pollination, the ovary in the gynophore elongates up to 27 cm and penetrates the soil up to 7 cm, and forms a pod that usually contains a seed (Balittan, 2004). The deeper the root area, the smaller the root density, the root zone with a large density occurs at 1–10 cm below the soil surface (Pronaningrum, 2016).

Arachnis pintoi can be propagated using seeds, cuttings, and stolon (Balittan, 2004). The germplasm of pinto peanut shows wide variability in adaptation, dry matter yield, nutrient content, and seed production (Carvalho and Quesenberry, 2012). Seed production studies of *A. pintoi* in Indonesia has not been widelycarried out because it takes about 8 months for pinto peanut to produce seeds, with a yield of 1.76 – 2.1 $ton.ha^{-1}$ (Fanindi et. al, 2012). Seed production of

A. pintoi increased with longer forage harvest time. The average forage production harvested at 6, 12, and 18 months was 1.8, 5.2, and 5.9 tonnes.ha^{-1}, respectively (Aminah et. al, 1994). Seed formation in the highlands can obtain seeds with high germination rates, whereas pinto peanuts grown in the lowlands produce fewer seeds with low germination rates (Neef et al., 2004).

The seeds of *A. pintoi* come from the gynophore extension, which forms a seed-filled pod (Figure 4). Seed formation can be promoted by gibberellic acid (GA_3) treatment; elevated levels of gibberellins can promote seed development, flowering, stem elongation, and leaf growth (Salisbury and Ross, 1995). GA_3 application to plants can increase the auxin biosynthesis through proteolytic enzymes that are formed and release tryptophan compounds as auxin precursors. Gibberellic acid (GA_3) can increase the percentage of flowers in pods (Salisbury and Ross, 1995).

GA_3 at a concentration of 80 to 160 ppm applied to peanut crops can prevent flower abscission, reducing flower drops and increasing pod production (Yennita, 2014). The formation of seeds in *A. pintoi* can potentially be increased by applying GA_3 exogenously to the plants. According to Putra (2012), the application of 100 ppm GA_3 can increase bulb production, the number of flowers, and the number of seed bunches of shallot "Super Phillips". A study in soybean by Irwan et al. (2019) reported that GA_3 application at 350 ppm increased the number of seeds. Seed production of *A. pintoi* is important for producing planting materials, particularly for the remote locations. Our current study provides information on the extent of GA_3 treatment for *A. pintoi* to produce viable seeds that are needed for propagation.

Material and Methods

The study was conducted at the Pasir Sarongge Experimental Field, University Farm IPB, Pacet District, Cianjur Regency, West Java, which has an altitude of about 1200 meters above sea level (m asl). The experiment was conducted from August 2020 - May 2021, during which the average rainfall was at 274.9 mm per month (BMKG, 2021). A single-factor randomized complete block design was used for the experiment which included five GA_3 concentrations, namely 0, 75, 150, 225, and 300 ppm (parts per million). An orthogonal polynomial follow-up test was conducted to determine the effective concentrations for GA_3 on seed formation and seed viability in every concentration with a significant or very significant effect. A plot of land measuring 2 m x 1 m was used with for planting material as many as 12 pols measuring 15 x 30 cm or comparable to a cover of 27% of the beds.

The experimental site was cleared, and a plot of beds measuring 2 x 1 m with 30 cm of distance between the plots and 40 cm of distance between replicates were prepared. The plot beds were treated with 2 tons.ha^{-1} of cow manure,1 ton.ha^{-1} agricultural lime,100 kg.ha^{-1} urea fertilizer,150 kg.ha^{-1} SP-36, and 150 kg.ha^{-1} KCl. Urea fertilizer was applied at the beginning of planting and 4 weeks after planting (WAP). Weed control was conducted manually throughout the study duration.

Varying concentrations (75, 150, 225, and 300 ppm) of GA_3 were prepared. For instance, 75 ppm of GA_3 was prepared by dissolving 75 mg GA_3 in 1 L of water. Plants were treated/sprayed with GA_3 at 30 DAP (days after planting) and repeated every 30 day-interval. A dose of 1000 L.ha^{-1} of GA_3 was usually given in the morning. Seeds from the plants were harvested after six months by disassembling the beds. After 1 month of storing the seeds, a seed nursery was prepared where seeds are sown on sand media that has been sifted and sterilized by drying in direct sunlight, followed by steaming for 1 hour, and redrying. Before sowing, the seeds were immersed in 1% KNO_3 solution for 2 hours to break seed dormancy.

Growth and production measurement data included the percentage of soil cover, the addition of new runners, flower production, harvested seeds, percentage of seed formation, seed weight, and seed germination percentage.

Results and Discussion

Growth and Acceleration of Soil Coverage After Application of GA_3

The acceleration of soil cover is an important aspect in developing *A. pintoi* as a bio mulch. The soil cover of 100% was achieved at 60 days after treatment (DAT) or 90 days after planting (DAP), at which time observations were completed. The acceleration of soil cover in all treatment plots did not vary, which means that the application of GA_3 had no significant effect on the acceleration of soil cover in all plots at 0, 15, 30, 45, and 60 DAT (Table 1). There were rapid growths during 45 DAT and 60 DAT, which significantly increased the percentage of soil cover.

Accelerated soil cover can be due to the rainy season, which promotes vegetative growth. Planting of *A. pintoi* in the wet or rainy season can increase plant growth, leading to the highest increase in

Table 1. The percentage of land coverage of *Arachis pintoi* in 0, 15, 30, 45, and 60 DAT.

Concentration of GA_3 (ppm)	The percentage of land cover				
	0 DAT	15 DAT	30 DAT	45 DAT	60 DAT
0	34.51	77.88	92.98	97.33	100
75	36.08	78.24	93.91	98.65	100
150	36.86	73.47	93.87	97.97	100
225	34.65	79.59	93.98	98.75	100
300	34.51	75.85	94.37	98.43	100
F-test	ns	ns	ns	ns	ns
KK %	12.42	7.29	3.00	1.22	0

Note: (ns) not significant according to test. DAT = days after treatment.

forage production, which is highly correlated with water availability (Fanindi et al., 2012). During the dry season, the production of *A. pintoi* tends to be lower (Fanindi et al., 2012).

The speed of land coverage by *A. pintoi* in our study is considered fast. A previous study conducted on the same bed area by Simbolon (2018) reported that 98% soil cover of *A. pintoi* occurred at 135 DAP, whereas Pronaninggrum (2017) reported 100% soil cover of *A. pintoi* occurred at 105 DAP. In this study, 98% of soil cover occurred at 75 DAP (60 DAT) and >100% at 90 DAP (75 DAT). The difference in land cover acceleration was thought to be caused by differences in location and location, climate, and soil conditions. Both previous studies were conducted in the lowlands. The soil analysis of the Pasir Sarongge experimental field had a C-organic content of 3.53%, compared to the 2.38% in the Pronaninggrum (2017) study. According to a study by Balittan (2004), *A. pintoi* can grow well in soils that have C-organic > 3%.

Arachnis pintoi Growth

The growth of new runners or individual plants is an important factor in accelerating land cover. The more *A. pintoi* individuals were formed, the faster the plant could cover an area. *Arachis pintoi* runners are stolon that has tap roots. The growth of new individuals can be determined by counting the final number of individuals formed at harvest. GA_3 resulted in a significant increase in the final number of individuals and the total new growth of individuals after 180 DAP (Table 2).

The orthogonal polynomial test showed that GA_3 concentration had a significant quadratic effect on the total addition of new individuals with the equation y = -0.0025x2 + 0.7094x + 58.336, and the minimum, optimum, and maximum concentrations were found, 137.88 ppm, 141.88 ppm, and 145.88 ppm (Figure 1). GA_3 application can increase the growth of new individual plants by 53.25% than the plot without treatment; application at 141.88 ppm can be done after 90 DAP or 60 DAT when the growth has reached 100% coverage to promote the vegetative growth before entering the generative phase.

Gibberellic acid (GA_3) application increased the number of *A. pintoi* individuals so that soil cover would be faster. Gibberellins regulate the process of plant growth and development which specifically plays

Table 2. Runner growth of *A. pintoi* at 180 DAP

Concentration of GA_3 (ppmP)	Runner growth	
	Initial (0 DAP)	Number of new individual plants (180 DAP)
0	50	59.25
75	44	89.75
150	53	126.75
225	50	76.00
300	48	52.50
F-test	ns	**
CV %	19.11	16.36

Notes: ** = very significant ; ns: no significant; DAP= days after planting

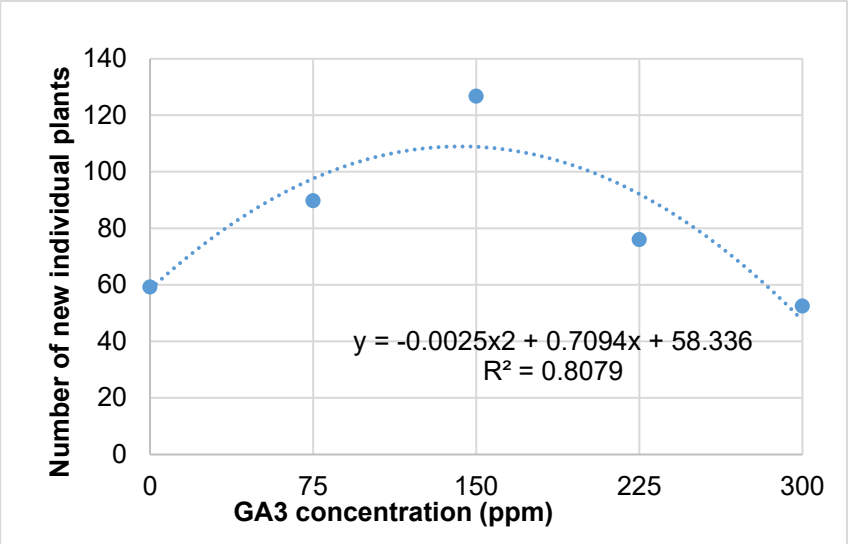

Figure 1. The orthogonal polynomial graph showing growth of new individual plants of *A. pintoi*.

an important role in plant stem elongation (Tiwari et al., 2011). *Arachis pintoi,* as a legume ground cover, can also be useful as a refugia plant and become a trap for natural enemies such as *Nepottetix apicalis, Nilaparvata lugens, Sogatella furcifera, Pachydiplosis oryzae,* and *Locusta migratoria*. Another study reported that planting A. pintoi as ground cover can increase the number of productive tillers in rice cultivation (Erdiansyah et al., 2018).

Effect of GA_3 on The Number of Flowers

The bright flower colour *of A. pintoi* is one of the factors that make it an attractive ornamental ground cover for home and public gardens. *Arachis pintoi* can flower throughout the year. The application of GA_3 stimulates to plant growth and development, such as the formation of flowers and fruit (Chen et al. 2014; Ramaiah et al., 2014). The application of GA_3 can increase production and the number of flowers formed in peanut plantations (Yennita, 2014). The number of flowers showed a significant difference after the GA_3 treatment entered the planting age of 135 DAT (Table 3).

GA_3 treatment did not affect the number of flowers per bed at 0, 15, 30, 45, 60, 75, and 90 DAT, but had a significant effect on the number of flowers per bed at the later stage of growth, i.e. 105, 120, 135, and 150 DAT (Table 3).

The results of the orthogonal polynomials analysis showed that the GA_3 treatment has a quadratic response to the number of flowers at 105 DAT (Figure 2) with the equation y = -0.0013x2 + 0.3398x + 104.51, and the minimum, optimum, and maximum concentrations were found, at 126.7 ppm, 130.69 ppm and 134.69 ppm, respectively. The quadratic response was also shown at 120 DAT (Figure 2) with the equation y = -0.0015x2 + 0.4115x + 105.14, and there were minimum, optimum, and maximum concentrations of 133.33 ppm, 137.1667 ppm, and 141.1667 ppm, respectively.

The further test of orthogonal polynomials at 135 DAT also showed a quadratic response to GA_3 application (Figure 2 C) with the equation y = -0.0014x2 + 0.3721x + 111.06 and the minimum, optimum, and maximum concentrations were 128.89 ppm, 132.89 ppm, and 136.89 ppm, respectively. The application of GA_3 at 150 DAT showed a quadratic response to the number of flowers (Figure 2 D) with the equation y = -0.0017x2 + 0.4526x + 116.86, and the minimum, optimum, and maximum concentrations were 129.11 ppm, 133.11 ppm, and 137.11 ppm, respectively. GA_3 at 150 ppm increased the number of flowers by 23.08 % to 28.59% than the plots without treatment. GA_3 application according to the optimum concentration of 130.69 ppm – 137.16 ppm can be conducted at 120 DAP or 90 DAT to increase flower production (Table 3).

The increase in flower formation of *A. pintoi* can be influenced by gibberellic acid, which has an important role at the initiation stage in flowers and at the early flower development stage, which allows GA_3 to influence cell differentiation (Yasmin et al., 2014). The results showed that a higher concentration of gibberellins increased the number of flowers formed (Table 3).

In addition to uses as ornamental ground covers, the yellow pinto peanut's attractive flower shape has insect-repellent properties. According to Kurniawati

Table 3. Number of *Arachnis pintoi* flowers per bed at 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, and 150 days after GA_3 treatment

Concentration of GA_3 (ppm)	Number of flowers per bed										
	0 DAT[t]	15 DAT[t]	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	105 DAT	120 DAT	135 DAT	150 DAT
0	14.50	37.50	101.50	145.00	128.75	109.75	116.00	110.25	109.25	116.00	123.25
75	16.75	36.75	89.75	130.00	145.00	130.00	121.25	104.00	112.75	114.00	124.00
150	16.00	38.50	77.00	137.25	143.00	123.50	134.50	149.50	153.00	156.50	160.25
225	17.00	35.00	70.00	115.25	134.25	104.00	99.50	106.50	110.25	111.75	132.25
300	8.00	36.00	82.00	140.25	132.75	110.50	112.00	95.00	96.25	97.75	98.50
F-test	ns	ns	ns	ns	ns	ns	ns	**	**	**	**
CV %	18.27	11.65	28.46	22.06	18.16	23.32	17.71	8.75	7.62	11.95	12.58

Note: ns=non-significant; (**) highly significant; DAT (Days after treatment). [t] data was transformed to Log(x) for analysis; the data presented is before transformation

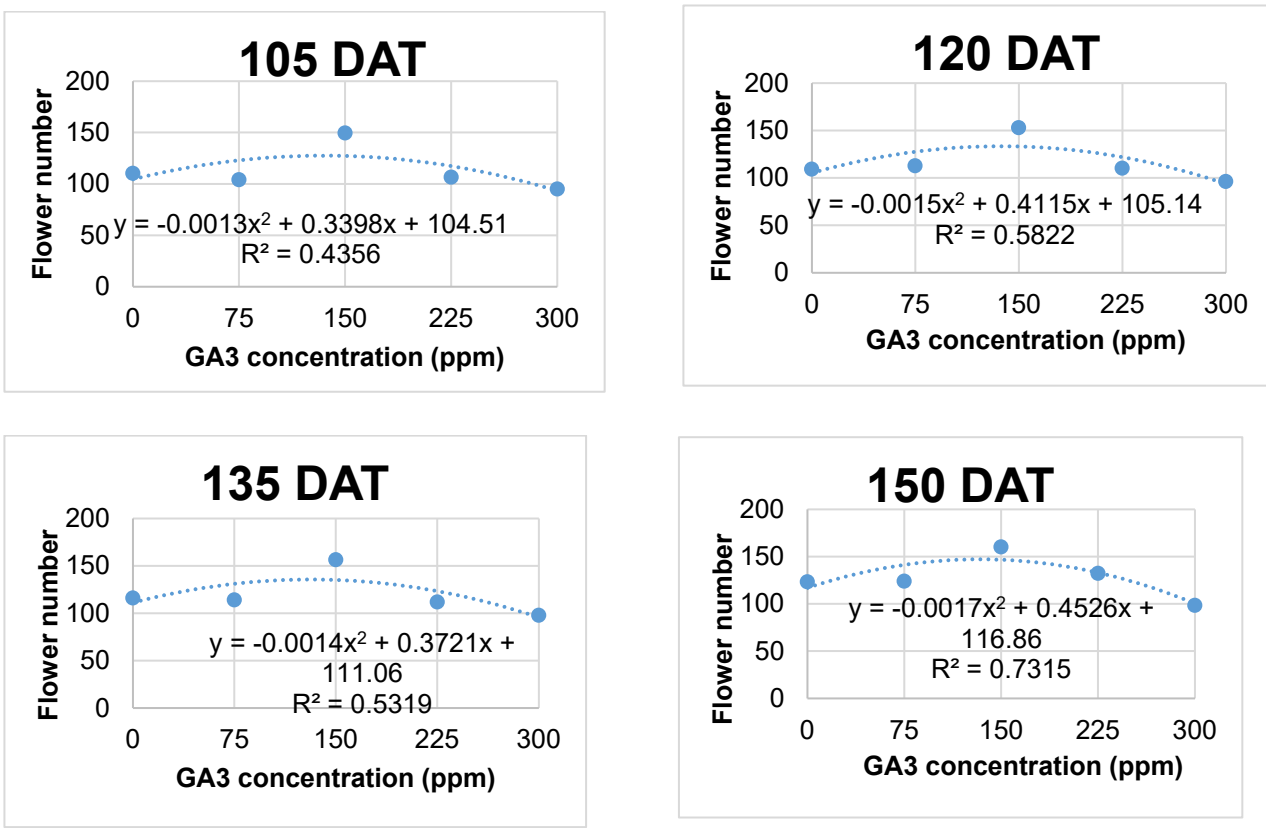

Figure 2. Orthogonal polynomial graph of the number of *A. pintoi* flowers per bed at 105, 120, 135, and 150 days after treatment (DAT)

and Martono (2015), insects prefer flowers that are small in size, have an open shape, and have a long flowering duration. Therefore, *Arachis pintoi* have these features that allow them to be used by insects as refugia plants. Refugia plants function as natural habitats, and alternative habitats for insects so they do not infest the main crops (Leksono and Yanuwiadi, 2013).

Effect of GA_3 Application on Seed Production

GA_3 application had significant effects on the total number of pods with a quadratic effect on the total number of pods harvested at 6 months (y = -0.001x2 + 0.2536x + 109.51) (Table 8, Figure 6). The minimum concentration was 122.8 ppm, an optimum concentration of 126.8 ppm, and a maximum concentration of 130.8 ppm (Figure 6). The optimum concentration of 126.80 ppm could be recommended when the plants are at 150 DAT or 120 DAP to increase the total number of pods harvested. GA_3 did not affect the number of intact, half-full, and empty pods at all concentration = (Table 4).

Arachnis pintoi pods are formed from gynophores that extend and penetrate the soil. The gynophores are derived from the extension of the flower stalk (Figure 4).

Figure 4. Elongated gynophores of *A. pintoi* that have formed pods

Harvested pods are classified into intact, half-full, and empty (Figure 5). In *A. pintoi*, intact pods fully contain seeds, half-full pods contain seeds that are slightly wrinkled and do not fill the pods, and empty pods do not contain seeds or small wrinkled seeds (Figure 5).

The yield of harvested pods of *A. pintoi* was dominated by empty pods, resulting in reduced seed production. The high number of empty and half-full pods could be due to insufficient photosynthates

distributed throughout the pods to form seeds. The assimilate is described by plant dry weight, which was similar between treatments. Empty pods can be caused by various factors, such as lack of Calcium absorption, which plays an important role in seed development, quality, and production (Gashti et al., 2012). Empty pods can also be caused by plant pathogenic nematodes, which are carriers of soil-borne fungi such as *Rhizoctonia solani, Sclerotium rolfsii,* Fusarium sp., and *Aspergillus* sp (Wicks et al., 2011).

Figure 5. Classification of pods of *A. pintoi* (A) intact pod, (B) half-full pod, and (C) empty pod

Table 4. Effect of GA_3 application on harvested seed yield at 150 days after treatment (DAT).

Concentration of GA_3 (ppm)	Quantity of harvested seed			
	Total	Intact	Half-full[t]	Empty[t]
0	113.75	33.25	36.50	44.00
75	109.75	39.50	20.75	49.50
150	139.00	34.75	39.50	64.75
225	110.25	36.00	29.00	45.25
300	95.75	33.00	26.50	36.25
F-Test	*	ns	ns	ns
CV %	19.31	11.75	12.82	11.57

Notes: ns = non-significant ; *= significant; [t] data was transformed to Log(x) for analysis; the data presented is before transformation

GA_3 treatment did not affect the yield of *A. pintoi* pods, as indicated by the results that were not different from the control treatment. The application of GA_3 in the range of 150 ppm increased the number of pods harvested by 18.16%. Based on the results, the higher the concentration, the lower the yield of the harvested pods. This may be because the high concentration of GA_3 used can inhibit plant growth. Administration of GA_3 by spraying in the study of Azizi et al. (2012) showed that the lowest yields of harvested soybeans treated with high concentrations of gibberellins at 200 ppm to 375 ppm. In this study, the application of GA_3 did not affect the number and percentage of intact and wrinkled seeds (Table 5).

The number of harvested pods was not positively correlated with the number of pithy seeds. The high percentage of wrinkled seeds could be due to the time of harvest being too early so that the seeds have not been filled to the maximum. In-ground peanut (*Arachis hypogea*) plantations where plants are harvested young, the percentage of wrinkled seeds is high (Rahmianna et al., 2007). Wrinkling and incomplete development of seeds can be caused by the less optimal intake of assimilates. When the rate of absorption of assimilates by the seeds is low, the filling time to develop intact seeds is high (Christian et al., 2016). The seed filling time can also be influenced by environmental factors, such as an increase in temperature that can increase the amount of assimilating, so it is necessary to determine the effective harvesting age related to the harvesting of *A. pintoi* seeds.

Effect of GA_3 Application on The Weight of Harvested Pods

The weight of the harvested pods was calculated based on the pods that had been through a 30-day storage, and the seeds had been dried before storage. Drying was conducted to reduce the moisture

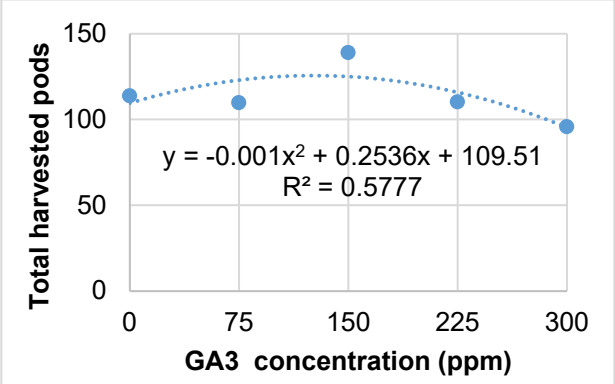

Figure 6. Quadratic orthogonal polynomial of the total harvested pods

Table 5. Quantity and percentage of intact and wrinkled seeds of *Arachnis pintoi* with and without GA_3 treatment.

Concentration of GA_3 (ppm)	Seed quantity		Seed percentage (%)	
	Intact[t]	Wrinkled[t]	Intact	Wrinkled
0	33,25	47,50	45,28	54,72
75	39,50	34,75	52,28	47,72
150	34,75	54,75	42,09	57,91
225	36,00	44,25	43,21	56,79
300	33,00	33,75	49,40	50,60
F-Test	ns	ns	ns	ns
CV %	11,75	14,46	20,97	18,19

Note: ns = non-significant; [t] data was transformed to Log(x) for analysis; the data presented is before transformation

content of the seeds so the seeds could be stored longer without rotting. The dry weight of the seeds is influenced by the remaining water content contained in the seeds (Kurniawan and Purnamwati, 2017). The weight of harvested seeds and the weight of 100 seeds were not affected by the use of GA_3 application after six months of planting (Table 6).

Similar seed weight between treatments might be due to the similar level of moisture content as a result of the drying and storage processes. During seed storage there can be a decrease in carbohydrate, lipid, and protein levels, which can affect seed weight loss (Begum et al., 2013). The weight of 100 seeds is also correlated with the shapes of the seed. Treatment with GA_3 at 150 ppm had a lighter 100-seed, so it can be estimated that this treatment resulted in more seed production than those of the other treatments.

Arachnis pintoi Seed Germination

The application of GA_3 did not have a significant effect on the percentage of germination (Table 7). Seed germination was carried out after the seeds were kept for 1 month of storage, because seeds from the Leguminosae, such as ground peanuts (*Arachis hypogea*) can have physiological dormancy after ripening. Seed dormancy is defined as the condition of seeds that do not grow or germinate within a certain period in favourable environmental condition (Widajati et al., 2013). The cause of physiological dormancy is that the embryo is not fully developed or immature (Hidayat and Wardiyati, 2019).

Breaking seed dormancy to increase germination can be done chemically, such as soaking the seeds in 1% KNO_3 for 2 hours immersion before the seeds were planted. This treatment can increase the absorption of water in the seeds (Candra et al., 2017) and stimulate enzyme activities related to germination hence enhancing seed germination (Hartawan, 2016).

Arachnis pintoi seeds can germinate 10-15 days (Balitan, 2004). Seeds treated with GA_3 at 150 ppm germinated of 76.25%, which is similar to the control treatment of 73.75%. These results agree with Neef et al. (2004) that *A. pintoi* seeds grown for six months in the highlands had a 68-73% germination rate. Seeds that do not germinate in our study might still experience seed dormancy, or the seeds have become non-viable. Seed dormancy can be caused by genetic factors, adverse environmental

Table 6. Weight of intact seed, wrinkles seed, and 100-seed weight.

Concentration of GA_3 (ppm)	100-seed weight (g)	Seed weight ($kg.ha^{-1}$)	
		Intact[t]	Wrinkled[t]
0	10.26	17.30	8.95
75	11.61	22.68	7.41
150	9.61	17.01	9.88
225	10.87	19.60	9.00
300	10.58	17.16	6.80
F-test	ns	ns	ns
CV %	7.02	14.00	22.12

Note: ns = non-significant; [t] data was transformed to Log(x) for analysis; the data presented is before transformation

Tabel 7. Percentage of seed germination

Concentration of GA_3 (ppm)	Percentage of seed germination				
	3 DAP[t]	6 DAP[t]	9 DAP[t]	12 DAP	15 DAP
0	22.50	27.50	41.25	48.75	73.75
75	23.75	30.00	35.00	43.75	68.75
150	18.75	26.25	37.50	51.25	76.25
225	13.75	15.00	21.25	33.75	57.50
300	17.50	20.00	26.25	35.00	57.50
F-Test	ns	ns	ns	ns	ns
CV %	24.02	22.68	14.20	28.25	19.83

Note: ns= non-significant. [t] analysis of variance was carried out on data that has been transformed to Log(x) and the data presented is data before the transformation. (DAP) Days after planting.

conditions, internal hormone imbalance, and physical barrier such as seeds having thick seed coats. Seed dormancy can also be related to unfavourable storage conditions and prolonged storage durations (Baskin and Baskin, 2014).

Conclusion

The use of the growth-regulating hormone GA_3 had a significant effect on the generative growth of *A. pintoi*; application of GA_3 at the optimum concentration range of 130.69 – 137.16 ppm increased the number of flowers at 105, 120, 135, and 150 DAT by 23.08% - 28.59%. GA_3 at 126.80 ppm increased the number of harvested pods by 18.16%, whereas GA_3 at 141.88 ppm increased the growth of new individual plants by 53.25 %.

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