An Investigation on Polyploidy Induction and Verification of Kram Ngo Plants (*Indigofera suffruticosa*) for Biomass Production in Northeast Thailand

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ABSTRACT

Two experiments were carried out at Sakon Nakhon Rajabhat University, Thailand during the 2015 aiming to induce more of polyploidy plants for biomass production by treated the two days germinated seeds of the Kram Ngo crop (*Indigofera suffruticosa*) with different levels of colchicine chemical, i.e. 00, 0.10, 0.20, and 0.40 % plus 6 and 12 hours soaking durations. With Experiment 1, it consisted of eight treatments and each was replicated 6 times. A Completely Randomized Design (CRD) was used. At fifteen days after germination, the seedlings were classified by naked eyes for percentages of abnormal and normal seedlings. At day 30 after germination, the seedlings were subjected to Flow Cytometry Analysis for an identification of polyploidy plants. With Experiment 2 at day 31, the Kram Ngo seedlings of diploid, mixoploid and tetraploid plants were transplanted into polythene pots and each of them was used as a treatment, i.e. three treatments were used. A CRD with 8 replications was used. The results of the Experiment 1 showed that 100% of normal seedlings were attained with T1 and T2 (control treatments, i.e. without colchicine chemical). It was found that soaking seeds of Kram Ngo crop for 6 hrs at a rate of 0.10% colchicine solution, it gave the most appropriate amount of polyploidy plants where it gave the percentages of 60 and 40 for tetraploid and mixoploid plants, respectively. Thus, colchicine chemical treatment significantly induced polyploidy plants of the Kram Ngo crop yet at a rate of 0.40% colchicine and treated for 12 hrs, it gave 100% diploid plants. The results of the Experiment 2 revealed that plant heights, numbers of compound leaves and numbers of branches of the tetraploid plants were significantly lower than that of the diploid plants but similar to the mixoploid plants. However, leaf areas of the leaflets of the tetraploid plants were highly significant over the control treatment (diploid plants). Similarly, Fresh weights of the leaflets of the tetraploid plants were also significantly higher than the diploid plants. Some important prospects in increasing biomass production were discussed.

Keywords: Diploid, mixoploid, tetraploid, Kram Ngo crop, *indigofera suffruticosa*, biomass production

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INTRODUCTION

In Thailand, particularly in northern and northeastern regions of the country, Kram Ngo crop (*Indigofera suffruticosa*) is natively known as indigo plants, it plays an important role in household economy since a large number of the villagers utilize natural dyeing substances derived from this crop plant for dyeing their homemade cotton materials as to attain a dark indigo color for their clothing materials. Nowadays, the products of cotton materials of its kind are insufficiently supplied in the local markets and overseas due to many factors and one of them is the supply of indigo dyeing substances derived from this particular plant which is not adequately available. Thus a large amount of the dyeing substances derived from the Kram Ngo plants are urgently needed by the local populations, yet the annually produced amount of the biomass has been inadequately met the demand of the villagers due to a small amount of the annual production of the biomass yielding which was relatively low since only 0.4 kg of dyeing substances could be taken from a 100 kg of the biomass fresh weight of the Kram Ngo plants (cf. Thailand Ministry of Social Development and Human Security). Therefore, some large amount of the biomass production of the Kram Ngo plants must be produced annually in order to meet a high demand of the dyeing industry. There are approximately 13 varieties of the Indigo plants locally available in Thailand (cf. Thailand Ministry of Agriculture and Cooperatives). The *Indigofera suffruticosa* plant is a self pollinated plant and it normally produces a large number of small individual flowers with a small size of individual seeds (3.40 – 16 g/1,000 seeds), hence it is rather sophisticated to carry out a normal breeding programme for further improvement of the cultivar. It has been advocated by some workers that tetraploid plants gave a larger top growth of biomass than its diploid progenitors of the same cultivar alone (Majdi *et al.,* 2010; Serapiglia *et al.,* 2014; 2015; Chen *et al.,* 2016). Furthermore, tetraploid plants possessed a high resistance to drought conditions, environmental stresses, insect pests and diseases (Predieri, 2001; van Laere *et al.,* 2011; Thong-on *et al.*, 2014; Serapiglia *et al.*, 2014; Zhang *et al.,* 2015; Tan *et al.*, 2015). Thus, this crop plant could be able to thrive on well under semi-arid conditions in Northeast Thailand.

In general, the methods in inducing tetraploid chromosomes of many plants could be carried out with the use of numerous methods, e.g. plant tissue culture, sterilized laboratory or even in a closed system glasshouse yet the induction of polyploidy plants could also be managed with the use of chemical substances such as oryzalin, trifluralin, amiprophos-methyl, N_2O gas and also colchicine (Blakeslee and Avery, 1937; Taylor *et al.*, 1976; van Tuyl *et al.*, 1992; Bouvier *et al.*, 1994). It is generally known that the use of colchicine gave better results than many other chemical substances and it is also known that colchicine is of alkaloid substance being extracted from the plants of meadow saffron (*Colchicum autumnale* L.). Colchicine normally retarded mitotic division of plant cells then polyploidy chromosomes production simultaneously took place (Planchiais *et al.*, 2000). A number of workers had used colchicine in different ways for their experimental treatments, e.g. with the use of plant buds (Chakraborti *et al.*, 1998; Abdoli *et al.*, 2013), with seedlings (Majdi *et al.*, 2010); with germinating seeds with the use of plain seeds (Grouh *et al.*, 2011; Blasco *et al.*, 2015). Surson *et al.* (2015) with *Citrus reticulate* Blanco also reported that germination of seeds, plant height, and numbers of leaves significantly decreased with colchicine chemical application. In some cases after colchicine treatment was carried out, some important recording parameters were used such as some records on morphological appearances of stomata, identification of polyploidy plants with the use of Flow Cytometer equipment (Chakraborti *et al.*, 1998; Majdi *et al.*, 2010; Abdoli *et al.*, 2013; Blasco *et al.,* 2015). The objective in carrying out this work lied mainly on the induction of polyploidy plants (diploid, mixoploid and tetraploid plants) of the Kram Ngo (Indigo plants) as to achieve

a greater biomass production of top growth of the crop plants so that some large amounts of dyeing substances derived from the biomass of the plants could possibly be attained. Thus this investigation may possibly provided some prospects on growth of this particular plant where it may be able to produce some large amounts of natural dyeing substances derive from top growth of the Kram Ngo plants hence the expansion of household textile industry with the use of cotton fibre materials may be successfully expanded, especially for the villagers in northeastern region of Thailand.

MATERIALS AND METHODS

Two experiments were carried out in the rainy season of the 2015 at the Faculty of Agriculture Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, Thailand. The works aim to provide information on both polyploidy induction and verification of the crop plants. Matured seeds of Kram Ngo legume crop (*Indigofera suffruticosa*) obtained from the Puparn Royal Development Study Centre, Sakon Nakhon Province, Thailand were used. For Experiment 1, the experiment consisted of four rates of colchicine solutions, i.e. 00, 0.10, 0.20 and 0.40% plus two soaking durations, i.e. 6 and 12 hrs. That is the first experiment consisted of eight treatments, and each treatment was replicated 6 times. Sixty fully matured seeds were used for each replication. A Completely Randomized Design (CRD) was used. Before the soaking of the germinated seeds was taken place, the seeds were kept in the fridge at 12°C for a few weeks before the starting of the experiment. The collected seeds were neatly cleaned by a tap water for 5 minutes followed by a 3-minute washing with the use of dish washing solutions then washed away twice by a tap water followed by a 10 minutes treatment in a 10% Clorox solution. This chemical was used to prevent the contamination of some diseases in seeds and then they were thoroughly washed with distilled water as to assure that the seeds were neatly cleaned. After the cleaning of the seeds was completed then the matured and fully filled seeds were selected and placed on tissue papers as to absorb away some amount of water surrounded the seeds then the seeds were placed into germinating wet towel paper in Petri-dishes. The germination of the seeds was carried out in the laboratory for 2 days and then all of the seeds were germinated successfully. At day 2 the germinated seeds were soaked with solutions of colchicine chemical (except those being used in the control treatments, T1 and T2) according to their respective treatments. The germinated seeds were transplanted in rows into the holes of the black plastic trays (60 plants for 60 holes of each tray) where the tray has a dimension of 55 x 36 cm in width and length, respectively. A single germinated seed was used for each hole of the tray where it contained a considerable amount of wet peat moss compost. The plants were allowed to grow for 15 days under field conditions then the seedlings were classified by naked eyes for normal and abnormal seedlings. The normal seedlings possessed straight, thinned and elongated hypocotyls and epicotyls with fully expanded leaves whilst abnormal seedlings possessed stunted growth with thick and short hypocotyls, and the growth of elongated epicotyls was relatively slow along with the appearance of curly leaves. Watering by a sprinkler irrigation system was carried out twice daily (at 9 am and 3 pm). At day 30 after germination, all of the seedlings of all treatments were subjected to Flow Cytometry Analysis system for its percentages of polyploidy seedlings, (diploid, mixoploid, and tetraploid chromosomes). The percentages of each type of the seedlings were recorded.

For the Experiment 2, the work was carried out with the use of the seedlings derived from the Experiment 1 when the seedlings reached 30 days after germination. The work consisted of three groups of the seedlings, i.e. diploid, mixoploid and tetraploid plants. Each of them was used as a treatment. The diploid seedlings treatment was used as a control treatment. A Completely Randomised Design (CRD) with eight replications was used. Individual plant seedling was carefully

transplanted into each polythene replicated pot at day 31 after germination. Each polythene pot has a diameter of 30 cm with a height of 23.5 cm. Each polythene pot contained nearly full amount of wet peat moss compost for use within the whole growing period of a 180-day after transplanted. Adequate water supply was carried out with the use of a sprinkler system and watering was carried out twice daily at 9 am and 3 pm to assure that the plants received adequate amount of water for the whole growing period. After the completion of the growing period of 180 days after transplanted, the plant samples were harvested. The following measurement parameters were used in order to attain mean values of each item. They include: plant height (cm, measured at approximately 1 cm above ground level up to the top), circumference of main stem (cm, measured at approximately 1 cm above ground level), numbers of nodes plant^{-1} and numbers of branches plant^{-1}. Other measurements parameters include numbers of compound leaves plant^{-1}, fresh weights of a compound leaf, numbers of leaflets within a compound leaf, length and width of a leaflet (cm), leaf index of the leaflets (Liu and Gao, 2007; Surson *et al.,* 2015) together with fresh weights (g) and leaf areas of the leaflets plant^{-1} (cm^2), and also leaflets fresh weights (g/cm^2 of leaf area). The attained data were statistical analysed where appropriate using a computer programme (SAS, 1998).

RESULTS AND DISCUSSION

RESULTS

Percentages of Abnormal Seedlings and Polyploidy Plants

For Experiment 1, the results being observed by naked eyes showed that abnormal seedlings were found with all of the colchicine treated plants but none was found with the seedlings of the untreated treatments (normal seedlings = thin seedling with lengthy stem). The values of the abnormal seedlings (abnormal seedlings = large short stems, i.e. the growth in girth) ranged from 20 to 67.27% for colchicine level of 0.40%, soaked for 12 hrs and the 0.40%, soaked for 6 hrs for T8 and T7, respectively (Table 1). For the percentages of the normal seedlings, it was found that 100% normal seedlings were found with those of the untreated seedlings (T1 and T2) whilst those treated with colchicine levels it gave the mean values ranged from 32.73 to 80.00% for colchicine level of 0.40%, soaked for 6 hrs (T7) and a level of 0.40%, soaked for 6 hrs (T8), respectively. At day 30 when the seedlings were identified with the use of a Flow Cytometry Analysis system, the results on the diploid % revealed that all of the untreated (control treatments, T1 and T2) seedlings gave 100% diploid plants whilst the highest level of the colchicine treatment (T8), i.e. 0.40%, soaked for 12 hrs gave the diploid plants of a 100% (Table 2). Whilst other treated treatments gave mean values of diploid plants ranged from 0.00 to 16.67% (T1, T2 and T6) for colchicine level of 0.10%, soaked for 6 hrs and colchicine level of 0.20%, soaked for 12 hrs, respectively. The results on the mixoploid plants showed that none of the mixoploid plants were found with those without colchicine treatments (T1 and T2). It was also found that the highest level of colchicine treatment, i.e. 0.40%, soaked for 12 hrs did not produce both mixoploid and tetraploid plants (T8) whilst the rest of the treated treatments gave a range of mean values of mixoploid plants of 33.33 to 66.67% (T6 and T5) for colchicine level of 0.20, soaked for 12 hrs and a level of 0.20%, soaked for 6 hrs, respectively. With the tetraploid %, a similar trend as that of the mixoploid plants was found with the tetraploid plants, i.e. none tetraploid plant was found with the treatments without colchicine chemical (T1 and T2) and also with the highest level of colchicine chemical, i.e. colchicine level of 0.40%, soaked for 12 hrs (T8). The rest of the treated plants gave values ranged from 27.27 to 60.00% for colchicine levels of 0.10%, soaked for 12 hrs (T4) and a level of 0.10%, soaked for 6 hrs (T3), respectively.

Table 1 Treatments (colchicine %), soaking durations, abnormal and normal seedlings of the Kram Ngo plants as affected by colchicine chemical levels and soaking durations

Treatments (colchicine %)	Soaking durations (hrs)	Abnormal seedlings (%)	Normal seedlings (%)
T1 (0.00, control)	0	0.00	100.00
T2 (0.00, control)	0	0.00	100.00
T3 (0.10)	6	43.64	56.60
T4 (0.10)	12	65.69	34.31
T5 (0.20)	6	55.56	44.44
T6 (0.20)	12	52.31	47.69
T7 (0.40)	6	67.27	32.73
T8 (0.40)	12	20.00	80.00

Table 2 Percentages of the polyploidy plants (diploid, mixoploid and tetraploid plants) as influenced by untreated and treated with different colchicine levels and soaking durations of the Kram Ngo seedlings, identified at day 30 after germination

Treatments (colchicine %)	Soaking durations (hrs)	Polyploidy plants of abnormal seedlings (%)		
		Diploid	Mixoploid	Tetraploid
T1 (0.00, control)	0	100.00	0.00	0.00
T2 (0.00, control)	0	100.00	0.00	0.00
T3 (0.10)	6	0.00	40.00	60.00
T4 (0.10)	12	9.09	63.34	27.27
T5 (0.20)	6	0.00	66.67	33.33
T6 (0.20)	12	16.67	33.33	50.00
T7 (0.40)	6	6.67	60.00	33.33
T8 (0.40)	12	100	0.00	0.00

Morphological Data of Kram Ngo Plants

With the results of the Experiment 2, it was found that plant height was highest with the treatment of diploid plants followed by that of the mixoploid plants and least with the treatment of the tetraploid plants with mean values of 76.17, 66.54 and 59.71 cm, respectively. The differences were large and statistically significant (Table 3). For the values of the circumference of stem plant^{-1}, the results showed that the diploid plants gave the highest value followed by the mixoploid plants and the lowest was with the tetraploid plants with mean values of 2.43, 2.18 and 2.32 cm, respectively. There was no statistical difference found among the three treatments. With the results on the numbers of the nodes, a similar trend as that of the circumference of stems was found, i.e. the highest value was found with the diploid plants followed by the mixoploid plants and least with the tetraploid plants with mean values of 35.25, 32.83 and 29.58 nodes, respectively.

There was no significant difference found among the three treatments. With branching numbers, it was found that the highest value was found with the diploid plants followed by the mixoploid plants and least with the tetraploid plants yet mixoploid plants were similar to that of the tetraploid plants with the mean values of 7.33, 4.58 and 4.25 branches plant^{-1}, respectively. The difference was large and statistically significant.

Table 3 Mean values of plant heights (PH), circumference of stems (CS), numbers of nodes (NN) and numbers of branching plant^{-1} (NB) of diploid, mixoploid and tetraploid plants of the Kram Ngo crop measured at day 180 after transplanted of seedlings

Polyploidy plants	PH	CS (cm)	NN	NB
Diploid plants	76.17^{a}	2.43	35.25	7.33^{a}
Mixoploid plants	66.54^{ab}	2.18	32.83	4.58^{b}
Tetraploid plants	59.71^{b}	2.32	29.58	4.28^{b}
F-test	*	ns	ns	*

Note: Letter (s) in each column indicated least significant differences at probability (p) < 0.05, ns = non significant

With numbers of the compound leaves plant^{-1}, it was found that the diploid plants attained the highest followed by the mixoploid and least with the tetraploid plants with the values of 79.42, 46.58 and 42.75 compound leaves, respectively. The differences were large and highly significant (Table 3). For fresh weights of the compound leaves, the results revealed that the tetraploid plants gave the highest followed by the mixoploid and least with the diploid plants. There was no statistical difference found among the three treatments used. With the numbers of leaflets within a compound leaf, the diploid plants gave the highest followed by the mixoploid and least with the tetraploid plants with mean values of 12.42, 11.42 and 10.67 leaves, respectively. There was no statistical difference found among the three treatments. The length of the leaflet was highest with the tetraploid plants followed by the mixoploid and least with the diploid plants. There was no statistical difference found among the three treatments used.

Leaf Index, Leaf Fresh Weight and Leaf Area

For mean values of leaf index, the results showed that leaf index was highest for the diploid plants followed by the mixoploid and least with the tetraploid plants with the mean values of 2.60, 2.20 and 2.05, respectively. The differences were large and highly significant (Table 4). With fresh weights of leaflets, a reverse result to that of the leaf index was found, i.e. the tetraploid plants gave the highest followed by the mixoploid and least with the diploid plants with the mean values of 0.050, 0.064 and 0.078 g, respectively. The difference was large and statistically significant. Leaf areas of the leaflets were highest with the tetraploid plants followed by the mixoploid and least with the diploid plants with values of 4.58, 4.03 and 3.25 cm^2, respectively. The difference was large and highly significant. With fresh weights of the leaflets/cm^2 of leaf area, the results showed that the fresh weights were highest with the tetraploid plants followed by the mixoploid and the lowest was with the diploid plants with mean values of 0.170, 0.128 and 0.015 g, respectively. There was no statistical difference found among the three treatments used (Table 5).

Table 4 Mean values of numbers of compound leaves plant^{-1} (NCL), fresh weights of a compound leaf (FWC), numbers of leaflets/a compound leaf (NLC), length of a leaflet (LL) and width of a leaflet (WL) of the Kram Ngo plants due to diploid, mixoploid and tetraploid plants

Polyploidy plants	NCL	FWC (g)	NLC	LL (cm)	WL (cm)
Diploid plant	79.42^{a}	0.807	12.42	3.45	1.33
Mixoploid plant	46.58^{b}	0.833	11.42	3.47	1.58
Tetraploid plant	42.75^{b}	0.911	10.67	3.62	1.78
F-test	**	ns	ns	ns	ns

Note: Letter (s) in each column indicated least significant differences at probability (p) < 0.05, ns = non significant

Table 5 Mean values of leaf index, fresh weights of leaflets plant^{-1} (FWL), leaf area of the leaflets plant^{-1} (LA), and leaflets fresh weights (LFW) of the diploid, mixoploid and tetraploid plants of the Kram Ngo crop

Polyploidy plants	Leaf index	FWL (g)	LA (cm^2)	LFW (g/cm^2 leaf area)
Diploid plant	2.60^{a}	0.050^{b}	3.25^{b}	0.015
Mixoploid plant	2.20^{b}	0.064^{ab}	4.03^{a}	0.128
Tetraploid plant	2.05^{b}	0.078^{a}	4.58^{a}	0.170
F-test	**	*	**	ns

Note: Letter (s) in each column indicated least significant differences (LSD) at probability (p) < 0.05, ns = non significant.

DISCUSSION

It was found that germination of seeds and the growth of seedlings of the Experiment 1 were normal for the control treatments (without colchicine), i.e. the seeds continued to germinate most rapidly with time and all of the germinated seeds produced normal appearance of both elongated hypocotyls and normal apical parts whilst that of the treated seeds gave a short bushy growth seedlings with an enlargement shape of stems and leaves, i.e. an increase in girth appearance of stems with a slow development of elongated epicotyls and a rounded shape of leaves when compared with that of the control treatments even though there was a relatively high percentage of germination of seeds (Surson *et al.*, 2015). The germinated seeds of the control treatments (without colchicine treatment) gave none of both abnormal seedlings whilst other treated germinated seedlings gave different percentages of the abnormal seedlings and the normal plants. The results indicated the influence of the colchicine chemical application where it affected or inhibited the growth of the germinated seedlings with their

physical appearances of somewhat abnormal compared with that of the control treatments. The percentages of the abnormal seedlings were highest with the treatment of 0.10% colchicine treated for 12 hrs where it gave 65.69% abnormal seedlings with normal seedlings of 34.31% yet with the level of 0.40% colchicine treated for 6 hrs it gave a slightly higher percentage of abnormal seedlings up to 67.27% and the normal seedlings of 32.73% only. It was also found that the germination of seeds was relatively low. The results indicated that a higher level of colchicine higher than 0.10% was not applicable for use due to the low percentages of the normal plants even though colchicine level of 0.4% gave up to 80% normal seedlings. This may be attributable to a large amount of the colchicine chemical (0.4%) received by seedlings did not affect numbers of polyploidy plants and most of the lower percentages gave a retarded feature in both the further germination of seeds and the growth of the seedlings. Although a slightly abnormal appearance of the seedlings was found in all colchicine treated seedlings yet there was no consistent trend found due to the effect of different levels of the colchicine chemical applications. The results indicated an optimum level of the colchicine to be applied, i.e. the treated level of 0.10% of colchicine treated for 12 hrs may be considered to be the best treatment since this level gave up to 65.69% of abnormal seedlings and 48.46% of the tetraploid plants. Thus the higher levels of colchicine, i.e. higher than 0.10 % largely inhibited the growth of the seedlings (Majdi *et al.*, 2010; Blasco *et al.*, 2015). Therefore, an induction of polyploidy chromosomes of the plants (diploid, mixoploid and tetraploid) of the Kram Ngo crop by colchicine chemical applications was successfully achieved, particularly with a colchicine solution level of 0.10% treated for 12 hrs where this treatment gave the highest percentage of the tetraploid seedlings.

The results of the Experiment 2 showed that plant heights, numbers of compound leaves and numbers of branches of the tetraploid plants were significantly lower than the diploid plants but similar to the mixoploid plants. The results indicated that colchicine had its significant effect on growth in height and numbers of branches of the Kram Ngo plants but not with the circumference of stem and numbers of nodes. The results indicated that the diploid plants could be able to produce a greater biomass production than the rest (mixoploid and tetraploid plants) yet none of the data on growth analysis with this particular crop, especially on the biomass of dry matter yields are available. Thus there is a need to carry out some growth analysis experiments of this particular crop in order to learn more of its partitioning of assimilates among the plant organs and nutrient manipulations on leaf growth must be taken into account. This is a way to attain more biomass of leaves and in return it could be provided a greater amount of dyeing substances for homemade textile industry. The results on fresh weights and leaf areas of the leaflets seem to provide some advantages in increasing biomass production since leaf index and leaf area of the tetraploid plants were highly significant over the mixoploid and the diploid plants. Furthermore, fresh weights of the attained leaflets were significantly higher than both the mixoploid and the diploid plants. The results suggested that there is a great tendency to increase biomass production of the Kram Ngo plants due to the reason that a compound leaf consisted of a large number of leaflets thus an increase in leaflets production by increasing number of leaflets in each compound leaf could presumably increase biomass production of the Kram Ngo plants. An increase in the thickness of the palisade and mesophyll layers of the leaflets may be attained by nutrients manipulation technique such as a slightly add up of the potassium fertilizer level to the soils greater than nitrogen as to gain more assimilates in leaves and stems may be massively contributed to the biomass production if soil pH value (1:2.5 soil : water by volume) could be remained in a range between 6 to 6.5 as to facilitate a rapid release of soil nutrients (Mengel and Kirkby, 1987; Suksri, 1998; 1999). Thus it may be possible that more of the dyeing substances could possibly be attained with a huge

contribution of leaflets within the compound leaves. Some further field experiments (not to be carried out in pots) on nutrients manipulation with the use of growth analysis technique (Sestak *et al.,* 1971; Suksri, 1999) may be urgently needed in order to understand the partitioning of the hydrocarbon of the assimilates among the different sinks/sources of the plants such as leaves, stems, fruits and others. Some records on dry matter yields plant^{-1} as a result of net assimilation rates of the plants may also be needed and also a high soil fertility level must be established in order to assure adequate amount of soil nutrients being released around the roots zone.

CONCLUSION

Polyploidy induction of diploid, mixoploid and tetraploid chromosomes in Kram Ngo plants (*Indigofera suffruticosa*) for biomass production with the use of colchicine chemical was possible and the most appropriate rate to be used is 0.10% and soaked in the solution for 12 hrs. It gave 65.69% of abnormal seedlings and 48.46% tetraploid plants and higher rates of the colchicine chemical higher than 0.10% retarded the growth of the germinated seeds of the Kram Ngo plants. Numbers of leaflets and leaf areas of the leaflets were significantly higher for tetraploid plants than both the mixoploid and diploid plants. The dyeing substances of indigo colour for dyeing of cotton materials derived from the Kram Ngo plants could be increased by increasing the biomass production of leaflets and leaf areas of each leaflet of the compound leaves plant^{-1}.

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