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# Plant growth regulator formulation for propagating Red Chili UNIB CH23 hybrid through stem cutting

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**Abstract** Vegetative propagation may serve as a sound practice to propagate hybrid chili with suitable formulations of auxins. Thirty five plant growth regulator formulas which combinations with the types and concentration of auxin plant growth regulator including Root Moise served as a control commercial formula were selected for growth enhancement testing of Red Chili cuttings. The results demonstrated that 26 out of 35 formulas induced rooting and shoot growth of chili cuttings, and 25 formulas promoted fruiting. Formula F22 (combination of 10 ppm IBA + 10 ppm NAA) was the best formula for the growth of UNIB CH23 hybrid chili stem cuttings. As compared to the control cuttings treated with commercial root growth regulator at 2 ml $L^{-1}$, plants from cuttings treated with Formula 22 grew 2 times greater shoot sprouts of 26.19%, and 36.54% greater number of shoots, 37.5% higher dichotomously branches and 4.83 times greater number of productive branches. In term of fruit production, plants from cuttings tretaed with Formula F22 were increased the number of fruits per plant as 1.93 times higher and the fresh fruit weight of 1.75 times higher than those control plants. Formula F22 can be used for promoting growth of vegetatively propagated elite red Chili UNIB CH23 hybrid from stem cuttings, might be used as a sound alternative for growing chili through generative propagation from seeds.

**Keywords:** Plant growth regulator, Red chili hybrid, Vegetative propagation, Auxin, Stem cutting

## Introduction

Red chili (*Capsicum annum* L.) is a very important agricultural commodity in Indonesia as most Indonesian cuisine uses chili as one of ingredients. However, chili price is very volatile markets ranging from Rp15.000 to Rp150.000 per kg, depending on the availability of the product, season, and others. The production of large fresh chili in 2014 was 1.075 million tons, being 61,730 tons (6.09 percent) greater than those produced in

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2013. This increase was due to an increase in productivity of 0.19 tons per hectare (2.33 percent) and an increase in harvested area of 4.62 thousand hectares (3.73 percent) as compared to 2013 (Central Bureau of Statistics, 2015). Chili has been cultivated including *Capsicum annuum, C. chinense, C. frutescens, C. baccatum, and C. pubescens*. Classification of species can be done by: morphological characters, especially flower morphology, crossing can be carried out between species, and hybrid seeds between fertile species (Pickersgill, 1989). Hybrid varieties are F1 generation crossed by a pair or more parents (pure lines) which have superior characteristics due to the heterosis phenomenon in the chili plants (Roy, 2000).

The superior Chilli hybrid UNIB CH23 being resistant to Begomovirus was created (Ganefianti *et al.,* 2010). In addition, CH23 hybrid chili has various advantages including fruit length, fruit diameter and fruit weight per plant which is higher than other UNIB hybrid varieties (Syukur *et al.*, 2010b). In addition, UNIB CH 23 hybrid chili is also very suitable to be planted in the lowlands and is able to produce and adapt well in organosol, ultisol and regosol soils (Ganefianti *et al.*, 2017). Chili hybrid has several advantages and disadvantages over those of open pollinated. The advantages included fast flowering and harvesting, highproductivity, big and long fruit with high-density and great fruit production per plant (Mopidevi *et al*., 2017). Whereas, the weakneses of hybrid chili may include require continuous crossbreeding techniques to produce hybrid chili seeds, a long time for production, and keep parents continuously. Hybrid chili planting can only be done one cycle, if the seeds produced by hybrid plants (F2) are replanted leading to the plant growth and yield not uniform because their genes will spread according to Mendel's law for F2 derivatives (Ganefianti *et al.,* 2017).

Vegetative propagation can be used to overcome such drawbacks. However, the research on vegetative propagation has not been interested by researchers (Sanatombi and Sharma, 2007; Shirai and Hamigori, 2004). There are several advantages, if hybrid chilies can be propagated using cuttings, including propagation of hybrid chili is not dependent on the seeds from the crossing of two compatible parents, plant is fast fruiting, propagule genetics will be the same as the parent, the method of seed preparation will be faster when compared to propagation through seeds (Ashrafuzzama *et al.,* 2009). In potatoes, the current plant propagation has been done by means of mini cuttings and is standardized to get a large number of mini tubers (Bisognin *et al*., 2015). The root induction of stem cuttings of *Malushu pehensis* includes four stages of root formation, namely root pre-emergence, early stage of root formation, massive root formation, and later stage of root formation (Wangxiang *et al.,* 2017). The use of plant growth regulators, especially auxin, is the common practice for root induction in propagation using cuttings in several plants, including Black Raspberry (Villa *et al.,* 2018), *Terminalia arjuna* (Babu *et al.,* 2018a), *Terminalia chebula* (Babu *et al.,* 2018b), Black pepper plant (*Piper nigrum* L.) (Sannidhi *et al.* (2018), Cavendish banana (Viehmannová *et al*., 2007), *Labisia pumila* (Hasan *et al.*, 2014), Henna (Sardoei *et al.,* 2013), and 12 citrus genotypes Sabbah *et al.* (1991), and *Rosa centifolia* (Akhtar *et al.,* 2015).

Research on vegetative propagation of chili using cutting has been reported both *in vitro* and *in vivo*. One *in vitro* study, Otroshy *et al.* (2011) stated that the best induction chili root on stem cuttings was produced in MS medium with the addition of 0.5 $mgL^{-1}$ Indole-3-butityc acid (IBA). Raj *et al*. (2015) reported that elongation of shoots and rootings of Naga chili (*Capsicum chinense* Jacq.) were produced on basal MS medium with the addition of 5 $mgL^{-1}$ BA and 0.5 $mgL^{-1}$ IAA. Whereas, *in vivo* study by Shirai and Hamigori (2004) proposed a method of vegetative propagation, using cuttings chili by soaking the stem tip cuttings at the length of 6 cm in a solution of 0.5% Indolebutyric acid (IBA), promoted root growth. The used parent plants are Chilli plants planted on hydroponic medium and there are no report on vegetative propagation of chili in soil media. Although, there are many advantages, the propagation of chilies using cuttings still needs to be studied for the roots induction of chili cuttings, and the life span of seedlings from fruiting of plant cuttings that may return to rejuvenation of plant from cuttings. For this reason, it is necessary to study of vegetative chili propagation using cuttings. The research objective was conducted to evaluate the type and concentration of auxin growth regulator for stimulating root growth of stem cuttings of a hybrid cultivar UNIB CH23.

## Materials and methods

The experiment was consisted of two stages using hybrid chili variety UNIB CH23. The first stage was to identify media composition suitable for chili growth to produce the greatest shoots/branches under greenhouse condition for the second stage experiment. The second stage was to screen auxin formula which is best for propagating chili stem cuttings.

The first stage was carried out in greenhouse at Faculty of Agriculture, Bengkulu University from November 2017 until March 2018. Seeds were sown using a tray for 4 weeks. Healthy seedlings were transferred to polybags (40 cm x 50 cm), filled with treatment media as in Table 1. A basic fertilizer, 2 $gL^{-1}$ NPK (16:16:16) was added. To prevent fruit fly attack 0.5 mg, insecticide Furadan 3G was added per planting hole. Plants were irrigated daily by watering up to field capacity. Weeds were manually controlled. Pests were sprayed with insecticides, Profenos and Imidachloride at concentration of 2g$L^{-1}$. Disease control was used 2.5 g$L^{-1}$ Mankozeb.

The experiment was consisted of 12 planting media compositions using ultisol top soil, river sand rice husk charcoal and composted cow manure (Table 1) and 5 replications of 4 sample plants each, with total of 240 plants. Treatments of media compositions were arranged under a Completely Randomized Design. The data were analyzed using a F-test of 5%. In significant data, difference between treatments were separated by using Duncan's Multiple Range Test (DMRT) at of 5%. Data were analyzed by CoStat Software version 6400 and Microsoft Excel 2010.

**Table 1.** Media Composition Treatment for CH23 Hybrid Chili Growth

| Treatments | Description |
|---|---|
| K1 | 100% Soil |
| K2 | 100% Sand |
| K3 | 50% Husk Charcoal: 50% Manure |
| K4 | 50% Soil: 50% Manure |
| K5 | 50% Soil: 50% Husk Charcoal |
| K6 | 50% Soil: 50% Sand |
| K7 | 50% husk charcoal: 50% sand |
| K8 | 40% Soil: 30% Husk Charcoal: 30% Manure |
| K9 | 40% Soil: 30% Sand : 30% Manure |
| K10 | 40% Soil: 30% Husk Charcoal: 30% Sand |
| K11 | 40% Soil: 30% Husk Charcoal: 30% Manure |
| K12 | 40% Husk Charcoal: 30% Sand: 30% Manure |

Note: composition was based on volume

The second stage was to evaluate a combination of auxin plant growth regulator (2,4-D, NAA, IBA), and concentration (0, 1, 5, or 10 ppm) as a rooting stimulant in UNIB CH23 Hybrid Chili. As a comparison, 2 g$L^{-1}$ Root Moise (RM) rooting stimulant sold in agricultural store was used. As a control, shoot cuttings were soaked in water. The cuttings used for the second stage were taken after the Hybrid Chili plants were fruiting and produced 5-7 dichotomous or at least 32 branches. Cuttings were soaked in one of 35 regulator treatments as shown in Table 2. Each treatment was repeated 5 times and each replication consisted of 3 cuttings, with a total of 525 cuttings. The experiment was performed in a Completely Randomized Design (CRD).

To maintain the freshness of explants, pots were covered with plastic cup for 6 weeks or until stem cuttings sprouted up to 70% of the total treatments tested. The sprouted cuttings were transferred into polyethylene bags with a size of 40 cm x 50 cm. During the growing period, plants were watered every day. Pest and disease control were done when symptoms are seen. Weed control was manually done. Vegetative phase was observed every week from cuttings sprouting up to 12 weeks after treatment (WAT). Generative phase was observed during the first harvest at 20 WAT.

**Table 2.** Treatments of auxin concentrations for cuttings of hybrid chili variety UNIB CH23

| Treatments | Description |
|---|---|
| F1 | without ZPT (control) |
| F2 (1D) | 1 ppm 2,4-D [1)] |
| F3 (1N) | 1 ppm NAA [2)] |
| F4 (1B) | 1 ppm IBA [3)] |
| F5 (5D) | 5 ppm 2,4-D |
| F6 (5N) | 5 ppm NAA |
| F7 (5B) | 5 ppm IBA |
| F8 (10D) | 10 ppm 2,4-D |
| F9 (10N) | 1 0 ppm NAA |
| F10 (10B) | 10 ppm IBA |
| F11 (1D + 1N) | 1 ppm 2,4-D + 1 ppm NAA |
| F12 (1D + 1B) | 1 ppm 2,4-D + 1 ppm IBA |
| F13 (1B + 1N) | 1 ppm IBA + 1 ppm NAA |
| F14 (1D + 5N) | 1 ppm 2,4-D + 5 ppm NAA |
| F15 (1D + 5B) | 1 ppm 2,4-D + 5 ppm IBA |
| F16 (1B + 5N) | 1 ppm IBA + 5 ppm NAA |
| F17 (5B + 1N) | 5 ppm IBA + 1 ppm NAA |
| F18 (5B + 5N) | 5 ppm IBA + 5 ppm NAA |
| F19 (1D + 10N) | 1 ppm 2,4-D + 10 ppm NAA |
| F20 (1 B + 10N) | 1 ppm 2,4-D + 10 ppm IBA |
| F21 (5B + 10 N) | 1 ppm IBA + 10 ppm NAA |
| F22 (10B + 10N) | 10 ppm IBA + 10 ppm NAA |
| F23 (1 D + 10N) | 5 ppm 2,4-D + 10 ppm NAA |
| F24 (10B + 5N) | 10 ppm IBA + 5 ppm NAA |
| F25 (10D + 10N) | 10 ppm 2,4-D + 10 ppm NAA |
| F26 (1D + 1N + 1B) | 1 ppm 2,4-D + 1 ppm NAA + 1 ppm IBA |
| F27 (1D + 1N + 5B) | 1 ppm 2,4-D + 1 ppm NAA + 5 ppm IBA |
| F28 (1D + 1N + 10B) | 1 ppm 2,4-D + 1 ppm NAA + 10 ppm IBA |
| F29 (1D + 5N + 1B) | 1 ppm 2,4-D + 5 ppm NAA + 1 ppm IBA |
| F30 (1D + 5N + 5B) | 1 ppm 2,4-D + 5 ppm NAA + 5 ppm IBA |
| F31 (1D + 5N + 10B) | 1 ppm 2,4-D + 5 ppm NAA + 10 ppm IBA |
| F32 (1D + 10N + 1B) | 1 ppm 2,4-D + 10 ppm NAA + 1 ppm IBA |
| F33 (1D + 10N + 5B) | 1 ppm 2,4-D + 10 ppm NAA + 5 ppm IBA |
| F34 (1D + 10N + 10B) | 1 ppm 2,4-D + 10 ppm NAA + 10 ppm IBA |
| F35 (2ml $L^{-1}$ RM) | 2 ml per liter of Root Moise |

[1] / 2,4-D)2,4-Dichlorophenoxyacetic acid), [2] / NAA)1-Naphthaleneacetic acid), [3] / BAP)6-Benzylaminopurine

Quantitative data were analyzed for variant by an F test at P = 5% level. When there was statistically differed in treatments, then Duncan's Multiple Range Test (DMRT) at level of 5 % was computed. Data were analyzed by CoStat Software version 6. 4 000 and Microsoft Excel 2010.

## Results

### *Plant media composition selection*

Hybrid chili UNIB CH23 seeds planted in various types of planting media compositions demonstrated the differences in their plant growth in plant height, dichotomous height, number of shoots and number of cuttings harvested from the plants (Table 3). Observation after transplanting for 12 weeks, hybrid chili plants growing on mixture medium consisted of 40% Soil: 30% Husk Charcoal: 30% Manure showed the best vegetative growth response. Four compositions of the planting media (50% Husk Charcoal : 50% Manure, 50% Soil: 50% Manure, 50% Sand: 50% Manure and 40% Soil: 30% Husk Charcoal: 30% Manure) were suitable for higher plant stature than other treatments. The highest dichotomous branches (35 - 37 cm) were produced in 50% Soil: 50% Manure composition. The greatest numbers of shoots (22 - 27 shoots per plant) were produced in media composed of 50% Sand: 50% Manure and 40% Soil: 30% Husk Charcoal: 30% Manure. Whereas, the greatest number of harvested stem cuttings for the first time (10 cuttings per plant) was produced from media composed of 40% Soil: 30% Husk Charcoal: 30% Manure.

**Table 3.** The effect of media composition on average value of the vegetative growth of Chili hybrid UNIB CH23 as source cutting material

| Parent Plants | Plant Height (cm) | Dichotomous Height (cm) | Number of Buds (cm) | Number of Cuttings per plant[1]) |
|---|---|---|---|---|
| K1 (100% Soil) | 39.0 cde | 30.0 b | 9.0 ef | 5.0 cd |
| K2 (100% Sand) | 37.0 de | 31.0 b | 4.0 f | 2.0 e |
| K3 (50% Husk Charcoal: 50% Manure) | **51.0 a** | 31.0 b | 19.0 bc | **9.0 a** |
| K4 (50% Soil: 50% Manure) | **49,0 ab** | **35.0 a** | 18.0 bc | 5,0 cd |
| K5 (50% Soil: 50% Husk Charcoal) | 42.0 cd | 31.7 b | 12.7 cde | 5,7 c |
| K6 (50% Soil: 50% Sand) | 44.7 bc | 30.7 b | 13.0 cde | 6.3 b |
| K7 (50% Sand : 50% Manure) | **50.7 a** | 30.7 b | **22,0 ab** | 6.7 bc |
| K8 (40% Soil:30% Husk Charcoal:30% Manure) | **52.0 a** | **37.0 a** | **27.0 a** | **10.0 a** |
| K9 (40% Soil: 30% Sand : 30% Manure) | 42.0 cd | 31.0 b | 17.0 bcd | 6.0 c |
| K10 (40% Soil: 30% Husk Charcoal: 30% Manure) | 44.3 bc | 30.7 b | 17.7 bc | 6.7 bc |
| K11 (40% Soil:30% Husk Charcoal: 30% Sand) | 41.0 cd | 30.0 b | 10.0 def | 3.0 e |
| K12 (40% Husk Charcoal:30% Sand:30% Manure) | 33.7 e | 27.7 c | 12.7 cde | 3.7 de |
| Deviation Coefficient | 7.89% | 4.37% | 20.88% | 18.45% |
| Test F level 5% | Significant | Significant | Significant | Significant |

Note: 1) the numbers of cutting were obtained from the first harvest. The values followed by the same letters in the same column are not significantly different in the DMRT Test of 5%.

### *Evaluation of plant growth regulator (PGR) composition formula*

Combination formulas of plant growth regulators resulted to differ in the percentages of hybrid chili UNIB CH23 cuttings that grew buds and produced fruits (Figure 1). Percentage of stem cuttings that grew, with an indicator formed 1 cm long shoots and at least 2 leaves within 2 weeks after planting (WAT), as much as 74.29 % or 26 PGR formulas. Formula F22 (10 ppm IBA + 10 ppm NAA) was able to grow hybrid chili stem cuttings reaching to 100%. It is interesting to note that not all of growing cuttings were capable of producing fruits. Only cuttings treated with 25 PGR formulas (71.43%), were capable of producing fruits, much lower than the number of live cuttings (from 26 PGR formulas), because plants in formula F27 (1 ppm 2,4- D + 1 ppm NAA +5 ppm IBA) which can grow to 50% but up to 12 WAT they did not bear flower and produce fruit.

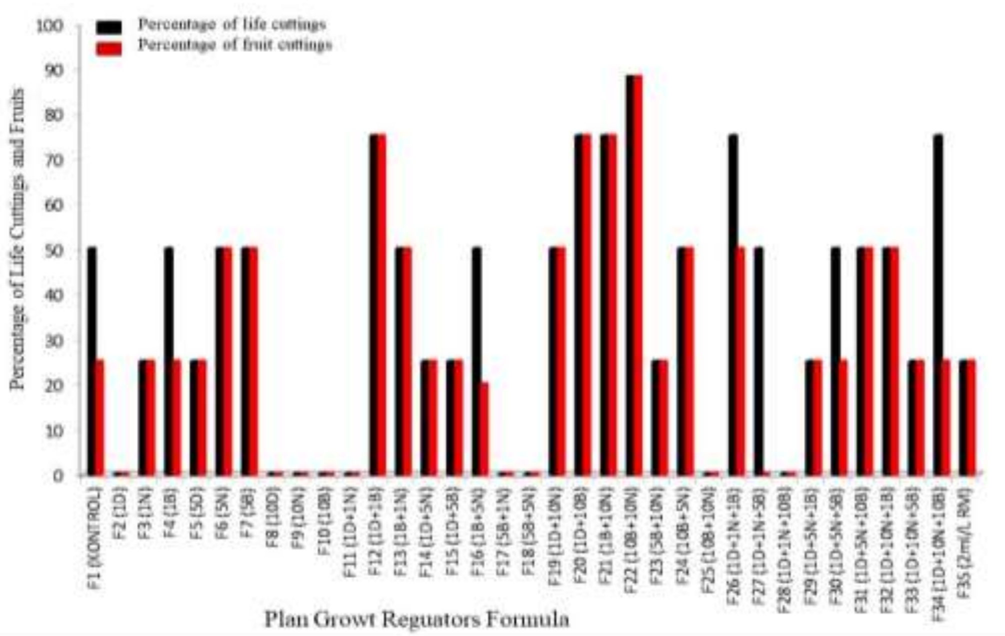


**Figure 1.** Response of life cuttings and fruits to plant growth regulator formula

Observation of the vegetative growth revealed that the variant analyzed results of the F test level 5% showed that the tested formulas of PGRs affected time to sprout, shoot height, number of shoots and productive branches of hybrid chili UNIB CH23 plants which originated from the cuttings at 12 weeks after planting, but did not affect the height of the dichotomous bud (Table 4).

Formula F22 (10 ppm IBA +10 ppm NAA), the percentage of hybrid Chili UNIB CH23 cuttings was able to grow and bear fruits reached to 100%. Soaking chili stem cuttings in Formula 22 was the best formula for the growth of UNIB CH23 hybrid chili stem cuttings. Formula 22 was able to grow shoot sprouts for 2 times, plant height 26.19%, the number of shoots 36.54%, dichrotomous branch height 37.5% and the number of productive branches per plant 4.83 times greater than the use of Root Moise, a commercial root growth regulator sold in the market, at 2 ml $L^{-1}$ (Table 4).

**Table 4.** The effect of growth regulator formula on vegetative growth of hybrids chili UNIB CH23 plants grown from cuttings at 12 week after treatments

| **Treatments [1/]** | **Time of Bud sprouting [2/] (WAT)** | **Shoot Height [2/] (cm)** | **Number of New Shoots [2/]** | **Dichotomous Shoot Height (cm)** | **Number of Productive Branches per Plant [2/]** |
|---|---|---|---|---|---|
| F1 (Control) | 8.67 c | 38.0 ab | 1 3.0 a | 2.5 | 7.1 bc |
| F3 (1N) | 6.00 b | 24.0 a | 22.5 bc | 2.0 | 5.3 b |
| F4 (1B) | 5.67 b | 31.0 a | 8.0 a | 2.5 | 4.0 a |
| F5 (5D) | 7.67 c | 29.5 a | 9.0 a | 2.5 | 6.3 bc |
| F6 (5N) | 6.00 bc | 40.0 ab | 11.0 a | 5.5 | 12.5 d |
| **F7 (5B)** | 4.67 ab | **51.0 cd** | **18.0 b** | **5,0** | **11.6 d** |
| F12 (1D + 1B) | 4.33 ab | 49.0 cd | 16.0 b | 4.5 | 8.8 c |
| F13 (1B + 1N) | 5.00 ab | 27.0 a | 9.5 a | 4.5 | 2.7 a |
| F14 (1D + 5N) | 4.00 ab | 44.0 bc | 12.0 ab | 4.0 | 9.4 c |
| F15 (1D + 5B) | 7.67 bc | 22.0 a | 14.0 ab | 5.0 | 2.6 a |
| F16 (1B + 5N) | 7.67 bc | 33.5 a | 34.0 d | 4.5 | 4.8 ab |
| F19 (1D + 10N) | 5.67 ab | 37.5 ab | 44.5 e | 5.5 | 9.2 c |
| **F20 (1B + 10N)** | 3.33 a | **42.0 b** | **31.5 d** | **5.5** | **13.3 d** |
| **F21 (5B + 10N)** | 3.33 a | **48.0 c** | **21.5 bc** | **3. 5** | **11.0 cd** |
| **F22 (10 B + 10N)** | 2.33 a | **53.0 d** | **33.5 d** | **5.5** | **13.5 d** |
| F23 (1D + 10N) | 5.33 b | 39.0 ab | 14.0 ab | 5.0 | 7.4 bc |
| F24 (10B + 5N) | 4.00 ab | 40.5 ab | 22.0 bc | 5.0 | 3.9 a |
| **F26 (1D + 1N + 1B)** | 2.67 a | **53.0 d** | **15.0 ab** | **3.0** | **5.3 b** |
| F27 (1D + 1N + 5B) | 7.00 c | 37.5 ab | 8.0 a | 3.4 | 5.8 b |
| F29 (1D + 5N + 1B) | 7.33 c | 28.0 a | 11.5 a | 5.0 | 2.8 a |
| F30 (1D + 5N + 5B) | 6.00 bc | 43.5 bc | 15.0 ab | 5.0 | 9.5 c |
| F31 (1D + 5N + 10B) | 5.33 b | 48.0 c | 17.5 b | 4.0 | 4.4 ab |
| F32 (1D + 10N + 1B) | 7.67 bc | 30.5 a | 20.0 bc | 4.5 | 4.5 ab |
| F33 (1D + 10N + 5B) | 5.33 b | 43.0 bc | 20.5 bc | 5.0 | 9.2 c |
| F34 (1D + 10N + 10B) | 6.67 bc | 37.5 ab | 20.5 bc | 4.0 | 6.7 bc |
| F35 (2ml / L RM) | 5.33 c | 42.0 b | 26.0 c | 4.0 | 2.8 a |

[1]/ D = 2,4-D (2,4-Dichlorophenoxyacetic acid), N = NAA (1-Naphthaleneacetic acid), B = BAP (6-Benzylaminopurine). [2]/ The numbers followed by the same letter in the same column are not significantly different in the DMRT test of 5% level. [2]/ WAT = week after planting

The plant growth regulation formula, and typical growth stages of hybrid chili UNIB CH23 plant which originated from stem cuttings consisted of stem base bulging, root emergence, root elongation and shoot growth (Figure 2). Hybrid Chili stem cuttings UNIB CH23 was induced by Formula 22 (10 ppm IBA +10 ppm NAA) that showed root formation and characterized by the base stem to swell (S0), starting from the second WAT. The roots began to grow about 2 mm (S1) in 3 WAT. At 4 WAT, the roots elongated reaching to 4.8 cm plus, 2 new roots were formed and buds had begun to grow in the stem node (S2). At 5 WAT, the longest root length reached 10.8 cm and the number of roots formed was 8 roots per cutting, the number of leaves formed was 10 leaves per cutting, and flowers began to form (S3) (Figure 2).


**Figure 2.** Stages of growth of UNIB CH 23 Hybrid chili shoot cuttings induced by Formula 22 (10 ppm IBA + 10 ppm NAA)

The use of plant growth regulator (PGR) formulas significantly affected the generative growth of UNIB CH23 hybrid chili grown from cuttings. The results of the F-test analysis at 5% level showed at 20 WAT. The treatment of growth regulating formula for soaking chili stem cutting base during propagation resulted in a significantly different on the number of fruits per stem, and the weight of hybrid chili fruit. The difference in the growth regulating formula tested did not affect the fruit diameter, fruit length and fruit weight per fruit (Table 5). Formula 22 (10 ppm IBA + 10 ppm NAA) was the highest significant increased the number of fruits per stem (13.5 fruits per stem). The weight of fruit in the first harvest at 12 WAT was 80.76 g per stem. The greatest number of fruits per stem (11.6 - 14.5 fruits per plant) was produced in Formula F5 (5 ppm 2,4-D), Formula F6 (5 ppm NAA), Formula F14 (1 ppm 2,4 - D + 5 ppm NAA, F20 (1 ppm IBA +10 ppm NAA), and Formula F22 (10 ppm IBA +10 ppm NAA). Fresh fruit yield of the first harvest at 20 WAT were high ranged from 69.83-80.76 g per plant that produced under Formula F14 treatment (1 ppm 2,4-D + 5 ppm NAA, F20 (1 ppm IBA + 10 ppm NAA) and Formula F22 (10 ppm IBA + 10 ppm NAA). The different treatments of growth regulating formulas did not significantly differed in the effect of the fruit diameter (5.5 - 8.5 mm), fruit length (12.8 - 15.5 cm) and fruit weight per fruit (3:48 - 5.98 g per fruit). Chili hybrid UNIB CH23 plants were propagated using stem cuttings with rooting stimulation by growth regulator substances F22 (10 ppm IBA + 10 ppm NAA) leading to increase the number of fruits per stem by 1.93 times, and the fresh fruit weight of the first harvest fruit at 20 WAT 1.75 times was higher than growth regulators sold in the market at 2 ml $L^{-1}$ Root Moise.

**Table 5.** The generative growth of stem plants of UNIB CH23 hybrid chili at 20 WAT

| **Treatment** [1/] | **Fruit Diameter** [/] **(mm)** | **Fruit Length (cm)** | **Number of Fruits per Stem** [2/] | **Fruit Weight per Fruit (g)** | **Fresh Fruit Weight** [2/] **(g)** |
|---|---|---|---|---|---|
| F1)Control) | 5.5 | 13.5 | 2.8 d | 3.48 | 9.75 e |
| F3 (1N) | 5.0 | 13.3 | 5.3 c | 4.23 | 22.42 d |
| F4 (1B) | 5.5 | 12.8 | 4.0 d | 5.21 | 20.85 d |
| F5 (5D) | 5.5 | 12.8 | 6.3 b c | 4.58 | 28.85 cd |
| F6 (5N) | 8.5 | 14.5 | 12.5 a | 3.52 | 43.98 bc |
| F7 (5B) | 8.0 | 13.5 | 11.6 a | 3.97 | 46.04 b |
| F12 (1D + 1B) | 7.5 | 14.6 | 8.8 b | 3.70 | 32.58 c |
| F13 (1B + 1N) | 7.5 | 13.8 | 2.7 d | 3.91 | 10.55 e |
| **F14 (1D + 5N)** | **7.0** | **15.0** | **12.5 a** | **4.82** | **69.83 a** |
| F15 (1D + 5B) | 8.0 | 14.3 | 2.6 d | 4.76 | 12.38 e |
| F16 (1B + 5N) | 7.5 | 14.2 | 4.8 cd | 4.34 | 20.85 d |
| F19 (1D + 10N) | 8.5 | 14.8 | 9.2 b | 4.42 | 40.70 bc |
| **F20 (1B + 10N)** | **8.5** | **15.1** | **13.3 a** | **5.31** | **70.58 a** |
| F21 (5 B + 10N) | 6.5 | 15.0 | 11 a b | 3.60 | 39.60 bc |
| **F22 (10 B + 10N)** | **8.5** | **15.0** | **14.5 a** | **5.98** | **80.76 a** |
| F23 (1D + 10N) | 8.0 | 14.5 | 7.4 b c | 3.95 | 29.20 cd |
| F24 (10B + 5N) | 8.0 | 14.8 | 3.9 d | 5.02 | 19.58 d |
| F26 (1D + 1N + 1B) | 6.0 | 13.9 | 5.3 c | 4.33 | 22.97 d |
| F27 (1D + 1N + 5B) | 8.0 | 14.0 | 2.8 d | 4.83 | 13.51 e |
| F29 (1D + 5N + 1B) | 8.0 | 15.5 | 9.5 b | 3.90 | 37.08 c |
| F30 (1D + 5N + 5B) | 7.0 | 14.3 | 4.4 c d | 4.27 | 18.78 d |
| F31 (1D + 5N + 10B) | 7.5 | 14.8 | 4.5 cd | 4.81 | 21.64 d |
| F32 (1D + 10N + 1B) | 8.0 | 14.4 | 9.2 b | 4.54 | 41.76 d |
| F33 (1D + 10N + 5B) | 7.0 | 14.2 | 6.7 b c | 4.61 | 30.88 cd |
| F34 (1D + 10N + 10B) | 7.0 | 14.2 | 7.1 c d | 3.35 | 23.79 d |
| F35 (2 ml $L^{-1}$ RM) | 7.5 | 14.8 | 7.5 b c | 4.83 | 46.24 b |

[1] [1]/ D = 2, 4 –D (2,4-Dichlorophenoxyacetic acid, N = NAA (1-Naphthaleneacetic acid), B = BAP (6-Benzylaminopurine). [2]/ The numbers followed by the same letter in the same column are not significantly different in the DMRT test of 5% level.

## Discussion

### *Plant media composition selection*

A good growing medium is the key to the success of multiplication the hybrid chili plants. The criteria for the parent plant can be considered as a source of cuttings include fast growth, large number of branches, high fruit production and disease free. The main target of the first stage was to gain a sufficient of shoots/branches as a source cutting materials for the second stage. Our experiment evaluated several natural materials, including husk charcoal, manure, soil, and sand which can be used as sources for planting media of chili. Results demosntrated that manure was the key material for combination with other materials for sufficient chili growth. The observation of the vegetative phase at 12 week after transplanting (WAT) demonstrated that population hybrid chili plants UNIB CH23, on medium mixture of 40% Soil: 30% Charcoal Husk: 30% Manure, generated the best growth response. In addition, our experiment revealed that any single material type produced unsatisfactory growth of chili. The compositions of three planting media 50% Husk Charcoal: 50% Manure, 50% Soil: 50% Manure, 50% Sand: 50% Manure and 40% Soil: 30% Husk Charcoal: 30% Manure were able to produce taller plant as compared to other treatments. The tallest dichotomies (35 - 37 cm) were produced from 50% Soil treatment : 50% Manure. The greatest number of shoots (22 - 27 shoots per plant) was produced by the treatment of 50% Sand: 50% Manure and 40% Soil: 30% Husk Charcoal: 30% Manure. In addition, the stem cuttings that harvested for the first time as many as 10 cuttings per plant was found from the treatment of 40% Soil: 30% Husk Charcoal: 30% Manure. Our results, that manure was important for the growth of chili, was supported by several researchers. Koshale *et al.* (2018) reported that Chilli plants grown on medium with the addition of NPK and manure (Poultry Manure) were able to produce better growth as compared on other treatments, especially for plant height, number of leaves, branches, flowers and fruits, fresh weight and dry weight of the plant. Likewise, Adhikari *et al.* (2016) reported that Compost and Poultry Manure gave the best results in accelerating the time of flowering, harvest time, stem diameter and height of sweet pepper. Manures are organic material which are mix between animal secretion (feces) with remaining feed, especially cow manure which was used in this experiment.

### *Formula for composition of root growth regulatory substances*

IBA and NAA both, singly or in combination are growth regulators which are widely used to stimulate the growth of roots of various types of plant cuttings, both for *in vitro* and for *in vivo* conventional propagation as being widely reported by several researchers. Akhtar *et al.* (2015) stated that 450 ppm IBA stimulate the roots of *Rosa centifolia* cuttings. Sabbah *et al.* (1991) demonstrated that 1000 ppm IBA + 3000 ppm NAA induce orange roots. Otroshy *et al.* (2011) reported that MS medium enriched with 0.5 $mgL^{-1}$ Indole-3-butityc acid (IBA) promote root formation and lengthening of chili stem cuttings *in vitro.* Our results demonstrated that Chili stem cuttings were induced to root *in vivo* by soaking the basal of the cuttings in Formula 22 (10 ppm IBA +10 ppm NAA). Root formation stages are as follows: basal stem begins to swell (S0) at 2WAT, roots begin to grow about 2 mm (S1) in 3 WAT, the roots elongated plus the formation of new roots and buds have begun to grow (S2) at 4 WAT, formation of massive fibrous roots, leaves, and formation of flowers (S3) at 5 WAT (Figure 2). The stages of hybrid chili root formation were similar to root induction process of stem cuttings of *Malushu pehensis* which included 4 stages of root formation, namely root pre-emergence (S0), early stage of root formation (S1), massive root formation (S2), and later stage of root formation (S3) (Wangxiang *et al.*, 2017).

Observation of the vegetative growth revealed that with Formula F22 (10 ppm IBA +10 ppm NAA), the percentage of hybrid chili UNIB CH23 cuttings which grew and bear fruit reached to 100%. The chili stem cuttings were soaked in Formula 22 (10 ppm IBA + 10 ppm NAA) showing the best formula to induce the vegetative growth of UNIB CH23 hybrid chili stem cuttings. Formula 22 was better than Root Moise at 2 ml $L^{-1}$ for growth of shoot sprouts, plant height, the number of shoots, dichotomic height and the number of productive branches per plant. This means that as Root Moise is general rooting PGR, it might not suitable for all plant species; whereas, Formula 22 was selected as the best for chili. Stem cutting has been used widely for vegetatively propagating many plant species both *in vitro* or *in vivo* with diverse use of auxin both in auxin type and in concentration. Villa *et al.* (2018) reported in Black Raspberry that basal cuttings produced the highest number of shoots, i.e. 1.3 shoots per cutting as compared to stem cuttings in the middle (1.1 shoots per cutting) and apical sections (0.7 shoots per cutting). The cuttings soaked in 2000 ppm IBA growth regulating agent produced 2 times greater rooting (52.2%) as compared to control cuttings (32.4%). Black Raspberry stem cuttings 10-20 cm length taken from the middle (middle) and tip (Apical) produced rooted cuttings as much as 48.3% (Apical) and 51.7% (middle), being better than the control (32.4%).

The application of growth regulators to promote the growth of *Terminalia arjuna* plant stem cuttings has been successfully carried out and produced a very significant response (Babu *et al.*, 2018a). The highest percentage of root formation (81.68 %), the greatest number of roots per cutting (20.41 roots per cutting), the longest root (9.53 cm), the greatest number of shoots (70.92 shoots per cutting), the longest shoot (31,9cm), the highest root weight (6.30 g) and highest shoot biomass (10.28 g) was generated by treatment of 2000 mgL$^{-1}$ IBA. The percentage of root number was higher as compared to stem cuttings soaked in ZPT solution 1,500 mgL$^{-1}$ IBA (61.37%), 2000 mgL$^{-1}$ IAA (70.34%) 1,500 mgL$^{-1}$ IAA (43.33%), and control, ie. stem cuttings which were only immersed using water produces the lowest rooted stem cuttings of 10.71% (Babu *et. al.*, 2018a). The effect of different concentrations of IAA and IBA ZPT with 0-2000 mgL-$^{1}$ treatment was also reported by Babu *et al.* (2018b) on *Terminalia chebula.* The success rate was lower than that produced in *Terminalia arjuna* plants. Concentration of 2000 mgL$^{-1}$ IBA was the best treatment and was able to have a significantly different effect from other treatments. The percentage of cuttings of *Terminalia chebula* rooted 61.74%, with 15.95 roots per cutting, root length 8.99 cm, sprouted percentage 68.11%, shoot length 21.23 cm, root biomass weight 4.63g and shoot weight 9.41 g. Sannidhi *et al.* (2018) reported that bush pepper is a miniature of the black pepper plant (*Piper nigrum* L.). Bush pepper plants are grown in decorative pots so that they have a higher economic value. Stem cuttings, taken from lateral branches (plagiotrops) of black pepper plants that have been fruiting, treated with one of 90 treatments, IBA, NAA and 2,4-D growth regulators with different concentrations were enriched with *Azospirillum lipoferum* and *Pseudomonas fleuroscen.* The results showed that the 1000 ppm IBA treatment was the best treatment for rooting being 65.30%, root fresh weight 1.10 g and root dry weight 0.53 g. The treatment could generate net income (Rs. 11.492.00 / 1000 cuttings) and produce the highest benefit cost ratio of 1: 3.43. Viehmannová *et al.* (2007) reported that the greatest number of roots and the best root growth of Cavendish banana plants were produced in MS medium with the addition of PABA (1 mgL$^{-1}$) + NAA (4 mgL$^{-1}$), whereas the greatest number of roots was produced with 5.4 μM Naphthaleneacetic acid (5.4 μM) seven weeks after subculture to rooting medium. Hasan *et al.* (2014) reported that the best adventitious root formation was produced by 5 mgL$^{-1}$ IBA on MS medium capable of producing roots as much as 72.4 ± 9.3%, 17.8 ± 9.4 roots per explant and 0.123 ± 0.096 g dry weight of the roots of the *Labisia pumila* plant. The best stem cuttings of Henna stem were produced by the treatment of 2000 mgL$^{-1}$ NAA combined with 200 gL$^{-1}$ salicylic acid by immersing the stem cuttings for 24 hours and then planted in sand medium (Sardoei *et al.,* 2013). Sabbah *et al.* (1991) reported that the roots of 12 citrus genotypes were successfully induced by the treatment of 1000 ppm IBA and 3000 ppm NAA as much as 70% of the total stem cuttings used. Akhtar *et al.* (2015) reported in *Rosa centifolia* that the concentration of 450 ppm IBA produced maximum shoot length (10.67 cm), the greatest shoot dry weight (3.02 g), the greatest root number (14.00), the longest root (11.90 cm) and the highest root dry weight 0.50 g per plant.

The success of rooting of stem cutting with the use of PGR Formula might have less significant meaning for chili as fruits that was the main harvested product for consumption. In perennial fruit trees, vegetative propagation such as cutting, in spite of for preserving genetic of the mother plant, has been commonly used for shorteing juvenile period of the plant. For this reason we also observed the generative growth and early yield of the chili following propagation by shoot cutting. Formula 22 (10 ppm IBA + 10 ppm NAA) was the best and significant formula for the number of fruits per stem and the weight of fruit in the first harvest. The PGR formulas did not affected fruit diameter, fruit length and fruit weight per fruit, as these characters are strongly regulated by genetics. It is interesting to note that PGR Formula F22 (10 ppm IBA + 10 ppm NAA) for rooting stimulant of stem cuttings of chili hybrid UNIB CH23 increased the number of fruits per stem and the fresh weight at the first harvest fruit than the use of Root Moise. The effect of growth regulators stimulating root growth does not occur directly. It is suspected that the proper growth stimulants can induce the formation and development of roots faster, more and longer. The good root system of the UNIB CH23 hybrid chili plant from cuttings can perform its main function in absorbing nutrients and water from the planting media needed by plants to support vegetative and generative growth. The diameter of fruit which ranged from 5.5 to 8.5 mm and the weight of fruit per fruit (3.48 - 5.98 g per fruit) of the UNIB CH23 Hybrid chili plant which was propagated using stem cuttings from this experiment, turned out to be comparable to those propagated using seeds (seeds) produced in an the experiment by Ganefianti *et al.* (2017) which was 7.9 mm and 5.71 g per fruit.

Ganefianti *et al.* (2017) tested that CH23 is one type of hybrid chili with high yield potential and well adapted to diverse altitude both the lowlands to the highlands, to various types of soil such as organosol, ultisol or regosol. Potential results when planted using seeds (seeds) are very important, among others, when the harvest is fast (69.3 days) and has a large fruit diameter (7.9 mm). Widyawati *et al.* (2014) and Syukur *et al.* (2010a) stated that the nature of fruit diameter is mainly due to genetic factors rather than environmental influences. Heritability estimates for chili fruit diameter is controlled by genetic factors reached 90.6% (Fitriani *et al.,* 2013 and Syukur *et al.*, 2010b). The response of CH23 hybrid Chili root cuttings growth with the addition of Formula F22 (10B + 10N) was an important finding in the vegetative propagation of hybrid chili. The results showed the regeneration ability of hybrid chili stem cuttings that matured to produce new plants and capable to produce fruits again. So far, generative propagation is a common method by crossing two or more selected parents to produce hybrid chili seeds. The discoveries that plant growth regulating substances stimulate root formation and elongation of cuttings might serve as one solution for farmers, because farmers can use stem cuttings / shoots of chili plants that are no longer productive as planting materials for cultivation of hybrid chilies. In other words, farmers do not always have to buy hybrid chili seeds for cultivation of hybrid chili.

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