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Effect of Basal Medium on *In Vitro* Leaf Morphology, Growth and Artemisinin Production of *Artemisia annua* L.

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Abstract
Artemisia annua L. was classified as one of the important medicinal plants due to its potential in the treatment of malaria. However, the propagation of this plant was limited by environmental and geographical factors. Therefore, *in vitro* culture technique was an alternative to overcome these limitations. Five different basal media were examined for their effect on the growth and artemisinin content of *in vitro* plantlets of *A. annua*. They were found to give different effect on the growth in term of height, fresh biomass and rooting ability of the plantlets. Glandular and non-glandular filamentous trichomes were observed on the adaxial and abaxial surface of *A. annua* leaf. The five basal media was found to affect the distribution and the number of trichomes and stomata formed on the leaf surfaces. LV medium induced more trichomes formation of both types on both leaf surfaces. Highest number of stomata was found on the leaf surface of the plantlets cultured in MS medium. While B5 medium resulted in non formation of stomata on the abaxial leaf surface of all the studied clones. Artemisinin production was found to greatly affect by the choice of basal medium used for cultivation.
Keywords: *Artemisia annua*, artemisinin, basal medium, growth pattern, stomata, trichomes

1. Introduction
Malaria is the leading causes of death globally especially in low-income countries (WHO, 2008). Artemisinin (qinghaosu) has become an important antimalarial drug especially when the *Plasmodium* species have developed resistance to the conventional treatment of quinine and chloroquine (Ferreira *et al.*, 2005). Artemisinin was found to be more effective compared to chloroquine, the most commonly used drug against the malaria parasite. *Artemisia annua* of the family Asteraceae remains the only botanical source for the production of artemisinin (WHO, 2001). However, there is a large variation in the production of artemisinin in the *A. annua* plants due to location and weather (Thu *et al.*, 2011). *A. annua* cultivation is restricted to temperate or high altitude regions. The production of artemisinin was found to be affected by temperature and altitude (Ferreira et al., 2010, Ivanescu et al., 2011). Limited cultivation, low content of artemisinin and the difficulty of biosynthesis of artemisinin lead to the commercial production of this drug uneconomical and therefore unable to meet the worldwide demands. To overcome this situation, *in vitro* culture techniques are promising alternatives for the propagation of elite clones with various optimizations for improving the artemisinin content in the *in vitro* cultures of *A. annua*. Many research reports had showed that the production of artemisinin was positively correlated with the number and types of trichomes produced from the *A. annua* plants (Duke, 1994, Ferreira and Janick, 1996, Lommen et al., 2006). Glandular trichomes of *A. annua* were confirmed to be the biosynthesis site of artemisinin and the abundance of glandular trichomes resulted in high artemisinin peak during flowering (Ferreira and Janick, 1996, Olsson et al., 2009, Duke, 1994). It was also reported that the artemisinin was mainly produced from the aerial parts of the plant (Ferreira *et al.*, 1995).
The growth of *in vitro* plants were significantly affected by the compositions of the basal medium (Gamborg *et al.*, 1976). Study had shown that the use of optimal basal medium enhanced the production of useful secondary metabolites (Ishimaru *et al.*, 1993). Hence, our study was carried out to investigate the effect of different basal medium on the morphology of the leaf trichomes, stomata and the growth of the *in vitro* plantlets of *A. annua* as well as establishing a correlation between the abundance of glandular trichomes with the artemisinin content of the *in vitro A. annua* plantlets.

2. Materials and methods
2.1 Plant material
Three different clones of *A. annua* of Vietnam origin, TC1, TC2 and Highland, were established from seeds on MS (Murashige and Skoog, 1962) gelled medium. The nodal segments (≈ 2.0 cm), excised from the eight weeks old seed-derived *in vitro* plantlets, were subsequently cultured on MS basal medium containing 30 g/L sucrose and 8 g of agar (Algas, Chile) for mass production of plant materials for the study. The *in vitro* plantlets were maintained under a constant temperature of 25 ± 2 °C with continuous lighting of approximately 32.5 µmol m^{-2} s^{-1} light intensity. The pH of all the culture media used in this study was adjusted to 5.75 before autoclaving (Tommy 325) at 121 °C for 11 minutes under 1.05 kg/cm^2 pressure.

2.2 Effect of basal medium on growth of Artemisia annua plantlets

The nodal segments of *A. annua* (TC1, TC2 and Highland) were cultured into five different hormone-free basal media: Murashige and Skoog (MS) (Murashige and Skoog, 1962), White (WM) (White, 1963), Litvay (LV) (Litvay *et al.*, 1981), Gamborg (B5) (Gamborg *et al.*, 1968), and Nitsch & Nitsch (NN) (Nitsch and Nitsch, 1969). Three explants were cultured into each 250 ml glass culture vessels containing 40 ml basal media supplemented with 3 % (w/v) sucrose and 8.0 g/L agar (Algas, Chile). Eight experimental units were used for each type of basal medium for each clone. The experiment was repeated four times. The experiment was carried out using complete randomized block design (CRBD) for each clone. The height of each plantlet of each clone cultured on different basal media was determined every week until the eight weeks culture period. After eight weeks of culture, all the plantlets of the different clone from the five different basal media were washed thoroughly and gently under running tap water to remove all adhering medium. Medium-free plantlets were blotted dry with kitchen towel, the fresh biomass of each plantlet and the length of the primary and secondary roots were determined. The data were analysed using Two-Way ANOVA and the best basal medium for each clone was determined using Tukey test at $p \leq 0.05$. Statistical analyses were performed using SPSS ver. 15.0 for Windows.

2.3 Effect of different basal media on leaf trichome and stomata of Artemisia annua plantlets

Leaves of three different clone cultured in different basal media were fixed in McDowell-Trump fixative prepared in 0.1 M phosphate buffer or cacoadylate buffer (pH 7.2) at 4 ^{o}C overnight. The leaves were then washed with 0.1 M phosphate buffer for 30 minutes and post fixed in 1 % Osmium tetroxide (OsO_4) solution at 25 ºC for 1 to 2 hours. The specimens were then rinsed with distilled water for 20 minutes and continued with dehydration process using 50 %, 75 %, 95 % and 100 % ethanol. The dehydrated tissues were immersed in hexathyldisilazane (HMDS) for another 10 minutes. HMDS from the specimen vial was decanted and the specimen vial with the tissue was left in the dessicator to air dry at room temperature. The specimens were then mounted with double adhesive tape on aluminium stubs, and coated with thin gold layer (40 to 60 mm) using Bio-Rad SEM coating system. Leaf morphology was examined with the aid of Scanning Electron Microscope (SEM) at 8 to 10 kV. The numbers of glandular and non-glandular trichomes and the length of glandular trichomes were determined using cell^B basic imaging software.

2.4 Extraction of artemisinin from in vitro A. annua plantlets

Dried plantlets of three different clones cultured in the five different basal media were separated into aerial part (stems and leaves) and roots. The dried aerial parts and roots (0.5 g and 0.1 g) were powdered with mortar and pestle accordingly. They were extracted with n-hexane and shaken for three hours on a gyratory shaker (ZHWY-3112, Shanghai). The collected supernatants were evaporated to dryness. Then, 1 ml of acetonitrile was added to the residue. The samples were then spotted on the TLC silica gel 60 F_{254S} plates (Merck, Darmstadt, Germany). The TLC tanks were presaturated overnight with n-hexane: ethyl acetate (8: 2) which was also used as the mobile phase. The plates were briefly immersed in a dipping reagent solution for coloration and visualization. The spots were allowed to develop on a stirring hotplate (Favorit HS0707V2) for a few minutes. The dipping reagent was made up of p-anisaldehyde (Acros organics), 95-97% sulfuric acid (HmbG chemicals), and acetic acid (HmbG chemicals) (0.5: 1: 50). TLC was carried out to confirm the presence of artemisinin in both parts of the plantlets before proceed to UPLC for quantification. The amount of artemisinin detected in the samples were quantify via ultra-performance liquid chromatography (Waters UPLC^{TM}) equipped with photo-diode array detector. The column used was Waters ACUITY UPLC^{TM} BEH column. The mobile solvent used in UPLC was consisted of acetonitrile and water in the ratio of 8:2 (v/v) with the flow rate of 0.25 mL/min.

3. Results and Discussion

3.1 Effect of basal medium on the growth of Artemisia annua plantlets

The growth of the *in vitro* plantlets of each clone was found to be different when cultured in different basal medium. B5 basal medium was found to be suitable for all the three different clones of *A. annua.* However, the responses to the other basal media were different in each clone. For the TC1 clones, the plantlet height was almost similar in all the five media between the 1st and 5th weeks of culture. The growth of TC1 plantlets was found to be not significantly different after eight weeks of culture on MS, B5 and NN medium. The growth of the *in vitro* plantlets was also found to be not significantly different when grew in NN, WN and LV media. Results obtained clearly indicated that MS and B5 supported better growth in term of plant height as compared to LV and WM media after eight weeks of culture (Figure 1a). While the growth of eight weeks old TC2 plantlets cultured in B5 was found to be not significantly different with plantlets cultured on LV and MS media which was also found to be not significantly different with NN medium. Medium WM supported the least growth for the TC2 plantlets and was significantly different with B5 medium (Figure 1b). Whereas the growth of Highland plantlets was significantly higher in when cultured in B5 as compared to MS, LV and WM media after eight

weeks of culture. The plantlets cultured on B5 and NN medium was also found to be not significantly different after the same growth duration. However, B5 supported better growth as compared to WM (Figure 1c). This was because the nutrients provided in each basal medium were different. Hence, selection of basal medium became important for efficient *in vitro* growth of the *A. annua* plantlets (Martin, 2004).

It was observed that the trend of morphological changes due to the five basal media was similar for each clone, TC1, TC2 and Highland. Each basal medium has its own specific effect on the plantlets morphology. TC1 was used as a model to describe the morphological changes due to the basal medium. MS medium supported a well balance growth with good aerial growth and the root system (Figure 2a). TC1 plantlets grew in B5 medium also produced good growth in aerial part but a very long primary root system (Figure 2b). This could be observed from the reasonably high total fresh biomass for the plantlets cultured in both basal media MS and B5 which was not significantly different statistically (Figure 3a). The WM basal medium only allowed poor aerial growth with thin and long primary roots (Figure. 2c). Again this was reflected in the low fresh biomass obtained from plantlets cultured in WM medium (Figure 3a). Plantlets cultured on LV medium produced more short shoot branching but with few roots (Figure 2d). NN medium supported long aerial part with no branching and short primary roots (Figure 2e). Even though the TC1 plantlets cultured in both these basal media showed different morphological characters, the total fresh biomass was not significantly different (Figure 3a).

Even though the five basal media have similar morphological effect on the in vitro plantlets of different clones, their effect on the fresh biomass was found to be different. For instance, plantlets of TC2 clones grew in MS, B5, LV and NN media were found with no significant difference in fresh biomass, in which 0.74 – 0.89 g of fresh biomass was produced in these four media. Significantly lower fresh biomass (0.20 g) was obtained from TC2 plantlets that were cultured on WM basal medium (Figure 3b). Those four types of basal media were also found to support not significantly different fresh biomass for the Highland plantlets. Only MS and NN basal media were found to be significantly better than WM basal medium for fresh biomass production. Total fresh biomass of Highland plantlets cultured in B5 and LV basal media was not significantly different from the WM medium (Figure 3c).

The effect on the plantlet morphology could be explained by the compositions of the basal medium. MS basal medium supported good growth was mainly due to the salt mixtures in MS that provide a balance and suitable nutrients for *in vitro* growth of most plant species (Chaturani *et al.*, 2006). In WM basal medium, nitrate (NO_3^-) is the only source of inorganic nitrogen. Nitrogen uptake is more efficient in the presence of both NH_4^+ and NO_3^- ions in the culture medium (George and Klerk, 2008). WM basal medium with just the presence of (NO_3^-) did not allow efficient uptake of nitrogen hence support poor aerial plant growth of *in vitro A. annua* plantlets. Additional supplied of glycine as nitrogen source in WM basal medium did not aid the growth of plantlets significantly. Therefore, dwarfism and stunted growth of plantlets in WM basal medium was possibly due to the insufficient uptake of nitrogen.

The function of myo-inositol is to control plant turgor pressure and protect plant from salinity stress (Nelson *et al.*, 1998). It was also found to be essential in synthesis of plant cell walls (Loewus and Murthy, 2000). WM basal medium was the only medium lacking of myo-inositol among the five basal media used in this study. This could be another factor that resulted in low biomass of the plantlets cultured in WM medium.

The high level of nitrate, potassium, phosphate and ammonium presence in all the basal media except WM explained the high growth of plantlets that resulted in higher fresh biomass in the other four basal media. The lower amount of these nutrients in WM hampered the growth thus produced plantlets with reduced fresh biomass. Phosphorus was found to be required for the cells to produce enough energy for cell division. Phosphorus deficiency in early stage of culture would decrease the metabolic rate and caused early senescence (Hasan *et al.*, 2010). For all the three clones, MS and B5 were found to support good growth and high biomass as the media contain high macro and micro nutrients suitable for the *in vitro* growth of a wide range of plant species (Narayan et al., 2005, Wang and Campbell, 2006).

3.2 Effect of basal media on rooting pattern of micro-shoots

It was observed that all the micro-shoots of the three different clones produced roots in all basal media after 7 days of culture. WM and B5 media supported good primary rooting for TC1 and Highland (Figure 4a and 4c). TC2 micro-shoots were able to produce primary roots in all basal media after eight weeks of culture except MS medium which support shorter roots and were are not significantly different with those found in LV and NN media (Figure 4b). B5 was also found to induce good secondary root growth for TC1 as compared to MS, LV and NN media (Figure 4a). While WM supported better secondary root growth of TC2 plantlets as compared to LV (Figure 4b). All the five basal support similar root growth for the Highland plantlets (Figure 4c). WM basal medium is the only medium supplemented with KCl, which provides more chloride ion (Cl^-). High Cl^- in the medium would act like auxin in promoting root formation and growth (Muniran *et al.*, 2007). Excess of NH_4^+ ions might inhibit root growth which was usually affected by the presence of NH_4^+ ions (Qin *et al.*, 2011). The low level of NH_4^+ ions in both B5 and WM basal medium explained the good rooting ability of all the three

clones in these media.

3.3 Effect of different basal media on trichome and stomata of Artemisia annua plantlets

Two types of trichomes (glandular and non-glandular filamentous) were found on adaxial and abaxial leaves surfaces of all three clones of *A. annua* plantlets cultured in all five basal media. Most of the non-glandular filamentous trichomes were observed in rows along the midrib of abaxial leaf surfaces (Figure 5a) and distributed randomly on adaxial leaf surfaces of *A. annua* (Figure 5b). Glandular trichomes were randomly distributed but not on the leaf midrib on both abaxial and adaxial leaf surfaces (Figure 5a and 5b). It was reported that *A. annua* plants grown in nature were equipped with two rows of filamentous and glandular trichomes (capitates trichomes) on adaxial leaf surfaces along the leaf midrib, whereas both the trichomes were randomly distributed on the abaxial surface (Duke and Paul, 1993). Therefore, it could be concluded that the distribution of glandular and non-glandular filamentous trichomes on the leaf surfaces of *in vitro* plantlets of *A. annua* and those grown in nature was different.

Number of trichomes and their length of *in vitro A. annua* plantlets were affected by the choice of basal media used for cultivation. Generally, plantlets cultured in LV basal medium produced more glandular trichomes on their leaf surface for all three clones if compared to plantlets cultured in the other four basal media. TC1 plantlets cultured in LV basal medium produced significantly higher number of glandular trichomes on abaxial leaf surface with 11.7 ± 2 trichomes in the area of 200 µm x 200 µm. Number of glandular trichomes observed on adaxial leaf surface of TC2 plantlets was 10.0 ± 2 (200 µm x 200 µm area) and was not significantly different from glandular trichomes produced on abaxial surface of TC1 plantlets. Highland plantlets cultured in LV basal medium were found to produce 8.0 ± 1 glandular trichomes on abaxial surface (200 µm x 200 µm area). Whereas significantly higher number of non-glandular filamentous trichomes was found on abaxial surface of TC1 plantlets cultured in LV medium (Table I).

NN basal medium was found to induce bigger glandular trichomes in adaxial surface of TC1 clones while the sizes of glandular trichomes in TC2 were not obviously affected by the different basal media (Table II). The adaxial surface of Highland clone induced the smallest glandular trichomes when cultured in WM medium. The presence of folic acid and biotin in the NN basal medium might be responsible for the growth of glandular trichomes.

Highest number of stomata were found on both abaxial and adaxial surfaces of TC1, TC2 and Highland plantlets cultured in MS basal medium (Table III). All the stomata were opened, because high humidity in culture vessel inhibited stomata closure (Roelfsema and Hedrich, 2005, Xie et al., 2006).

Interestingly, there were no stomata observed on abaxial leaf surface of all three clones cultured in B5 basal medium. Highland clone of plantlets cultured in WM basal medium were found with no stomata on abaxial leaf surface. TC2 plantlets cultured in LV and NN basal media were with the lowest number of stomata and none found on adaxial leaf surface respectively (Table III).

3.4 Correlation between number of trichomes and artemisinin content as affected by the basal media

Glandular trichomes were reported and confirmed to be the site of artemisinin production and most of these trichomes were found on the *A. annua* leaves (Ahmadi et al., 2002, Duke, 1994, Duke and Paul, 1993). Higher artemisinin content was reported to be present in the leaves and flowers of *A. annua*, only trace amount was found in the stem and none in the roots (Mannan *et al.*, 2010). Glandular trichomes were also found to develop rapidly on leaf primordial (Duke, 1994, Duke and Paul, 1993). Therefore the quantification of glandular trichomes on stems was not carried out as it has no significant affect on the artemisinin content.

Results obtained indicated that artemisinin content from the aerial parts of the plantlets cultured in five different basal media was not correlated with the number of glandular trichomes found on the leave surfaces but it was affected by the choice of basal medium used to culture the plantlets. However, the correlation of the abundance of glandular trichomes with artemisinin content was observed in plantlets cultured on WM and LV basal media. Artemisinin content in plantlets cultured in LV basal medium was reasonably high as they produced the highest number of glandular trichomes (regardless of leaf surfaces). While lowest artemisinin content was quantified in all clones of plantlets cultured in WM basal medium, as plantlets cultured in this basal medium was found with the least number of glandular trichomes. Even though plantlets cultured in B5 basal medium observed with few glandular trichomes only, but the percentage of artemisinin detected was high. This might due to the interactions of the plantlets to the composition of B5 basal medium or changes of culture environment. Similar trend was observed in the three studied clones of *A. annua* (TC1, TC2 and Highland) (Figure 6). Genetic and environment changes could be the factors for the absence of trichomes and artemisinin (Mannan *et al.*, 2010). To date no information can be obtained to indicate the positive correlation of artemisinin content to the size of glandular trichomes. Based on our result, it was clearly indicated that there was no correlation between the size of the glandular trichomes (Table II) with the synthesis and content of artemisinin (Figure 6).

4. Conclusion

LV basal medium was capable of supporting plantlets with more shoot branching but poor root system. The number of glandular trichomes on the leaves of *A. annua* plantlets cultured in LV medium was positively correlated with the production of artemisinin. Whereas *A. annua* plantlets cultured on B5 basal medium grew faster and produced high artemisinin content even though the number of glandular trichomes was few. MS basal medium supported plantlets with healthier aerial parts but only moderate artemisinin content. NN and WM basal medium were found to be not suitable for *in vitro* cultivation of *A. annua.*

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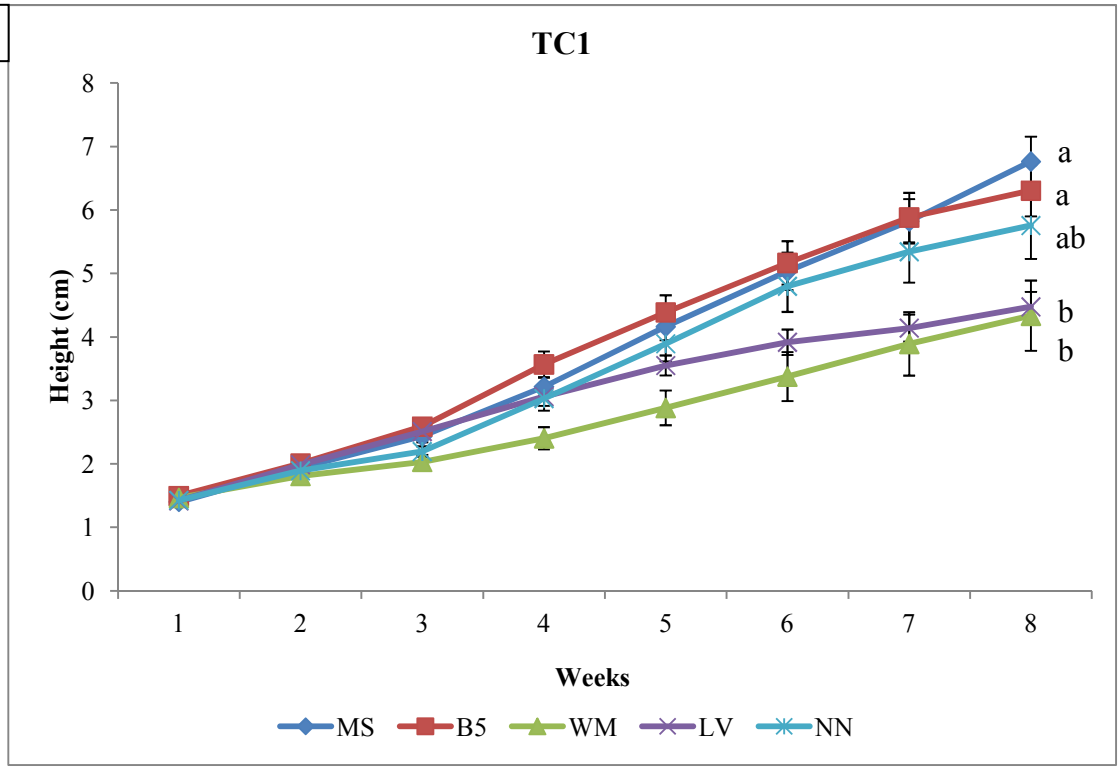
a



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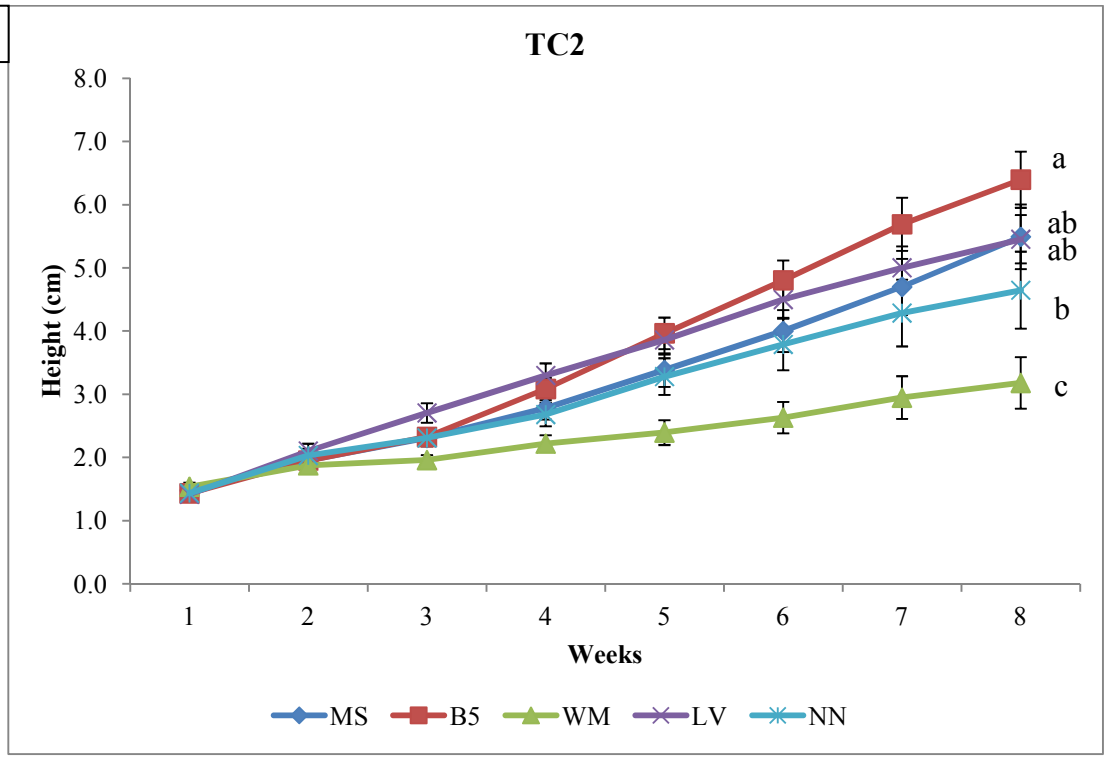

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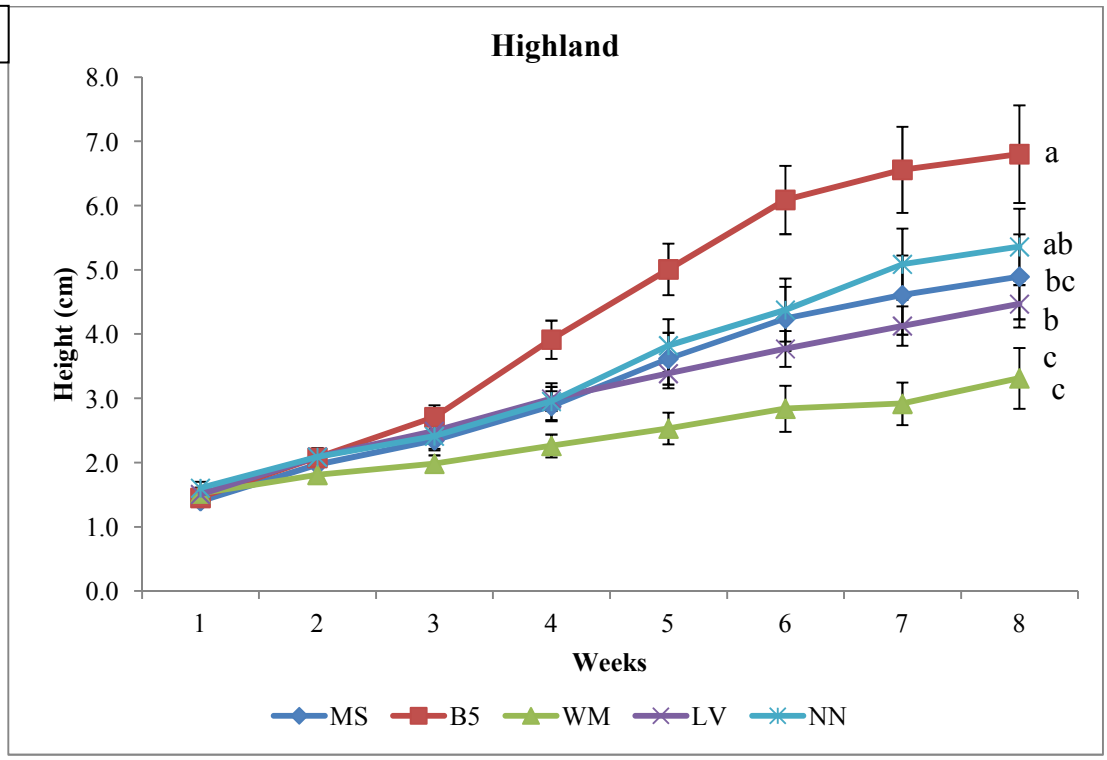
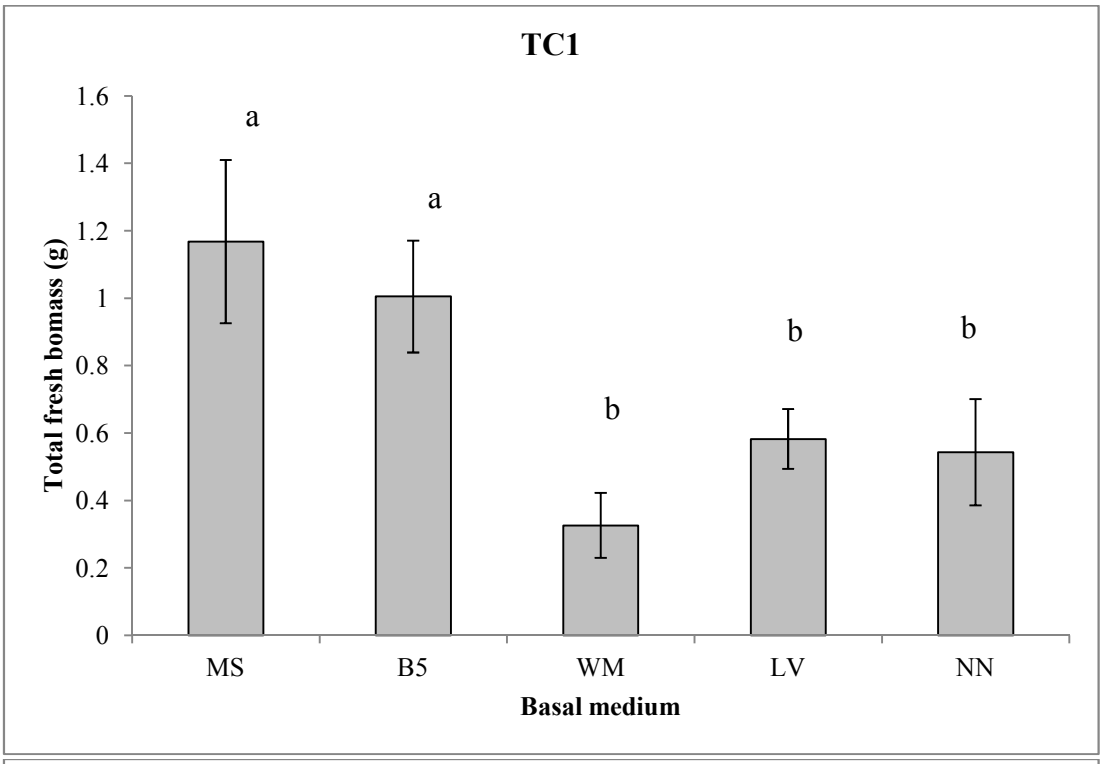


Mean values at week eight followed by the same alphabet were not significantly different (Tukey, $p \leq 0.05$). Bars represent mean ± standard error.

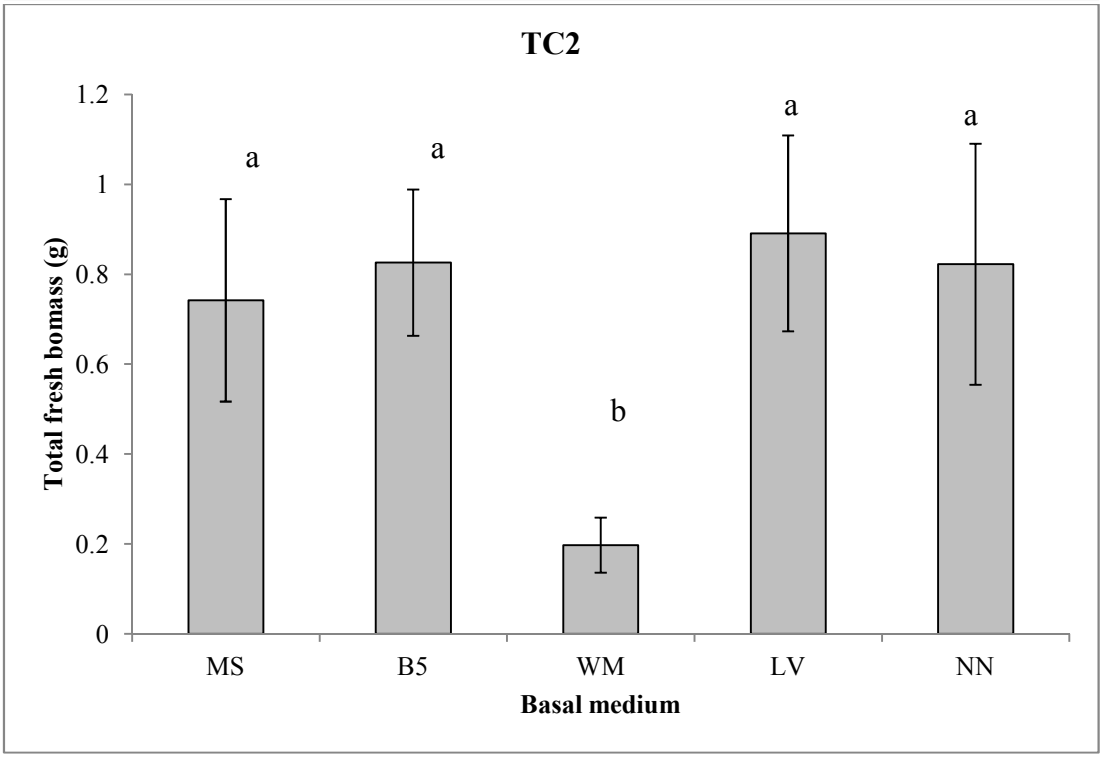
Figure 1. Growth pattern of different clones *A. annua* plantlets (a) TC1 (b) TC2 and (c) Highland cultured in five different basal media. (MS - Murashige and Skoog, B5- Gamborg, WM- White, LV- Litvay, NN- Nitsch and Nitsch)

Figure 2. TC1 plantlet in (a) MS basal medium (b) B5 basal medium (c) WM basal medium (d) LV basal medium and (e) NN basal medium.

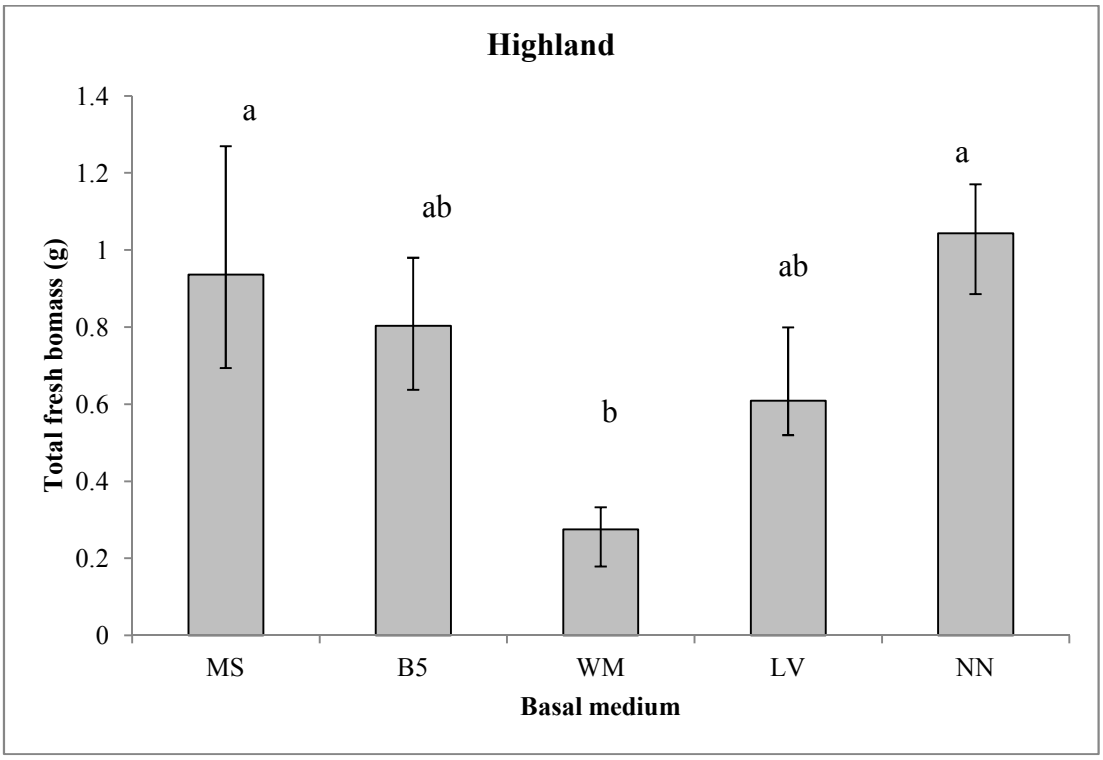
a


TC1
Total fresh bomass (g)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
b
b
MS
B5
WM
LV
NN
Basal medium

b


TC2
Total fresh bomass (g)
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
a
a
MS
B5
WM
LV
NN
Basal medium

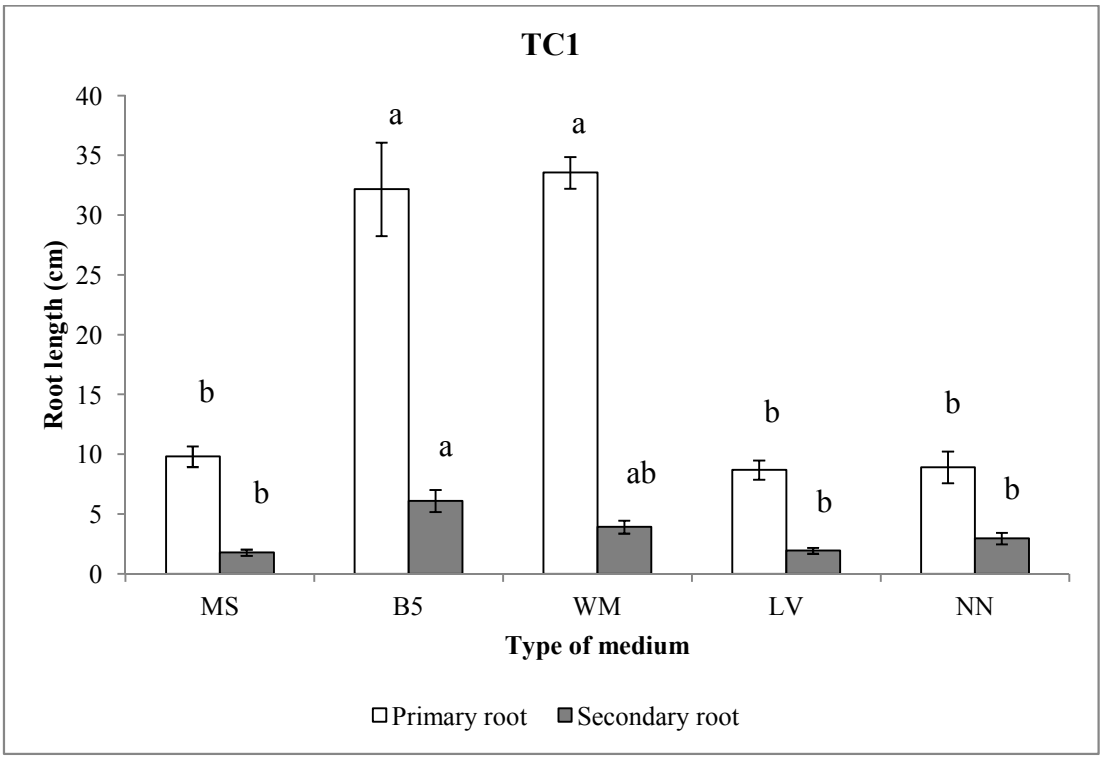
c



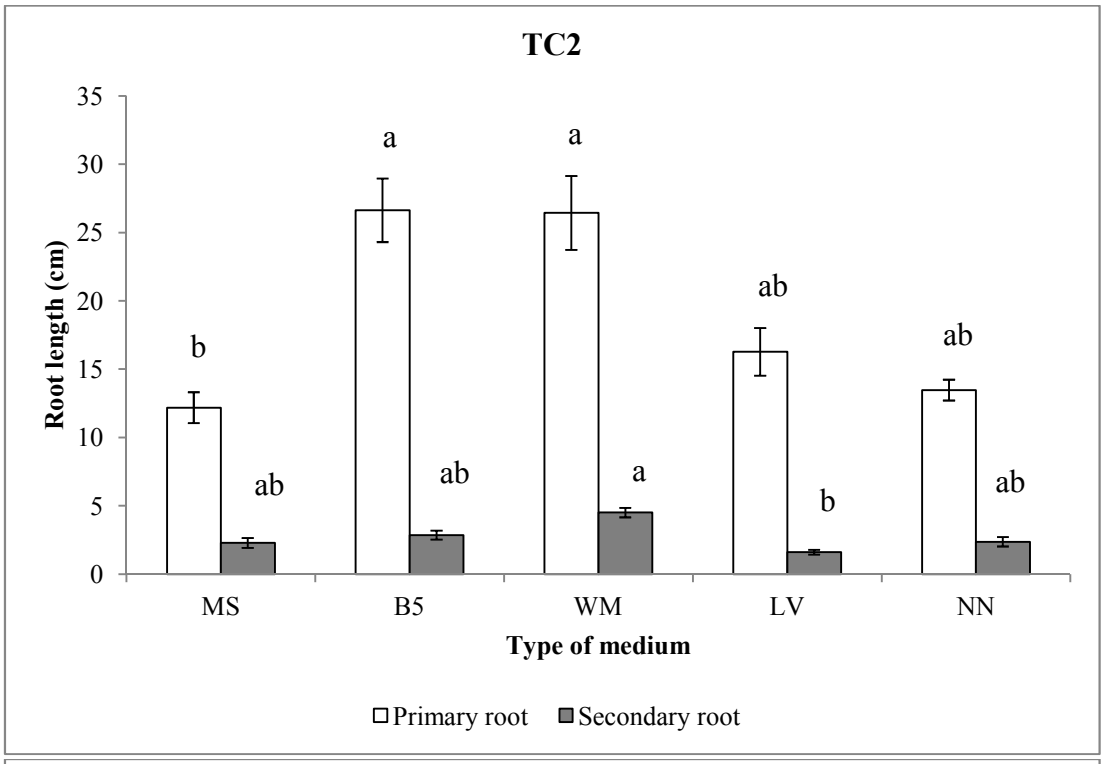
Mean values for each clone in different basal medium followed by the same alphabet were not significantly different (Tukey, $p \leq 0.05$). Bars represent mean ± standard error.

Figure 3. Total fresh biomass (aerial and root) of (a) TC1 (b) TC2 and (c) Highland clone of *A. annua* plantlets after eight weeks of culture.

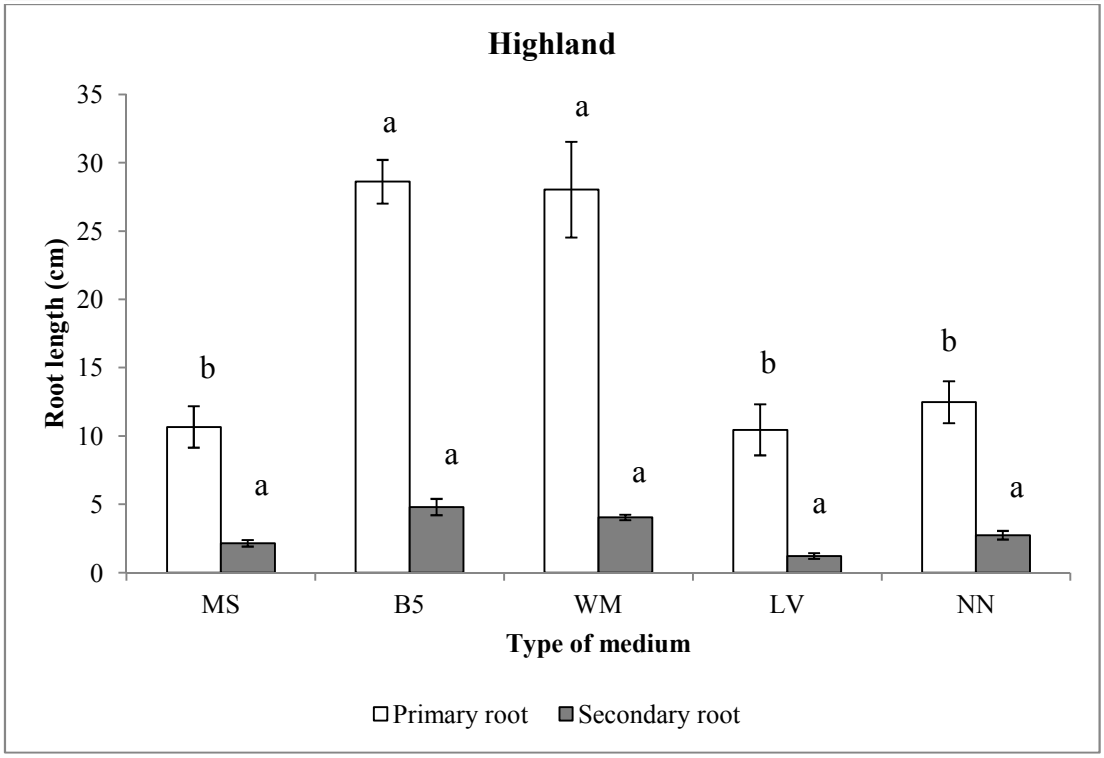
a



b



c



Mean values for each clone for each root type in different basal medium followed by the same alphabet were not significantly different (Tukey, $p \leq 0.05$). Bars represent mean ± standard error.

Figure 4. Length of primary and secondary roots of (a) TC1 (b) TC2 and (c) Highland clone of *A. annua* plantlets after eight weeks of culture in five different basal media.

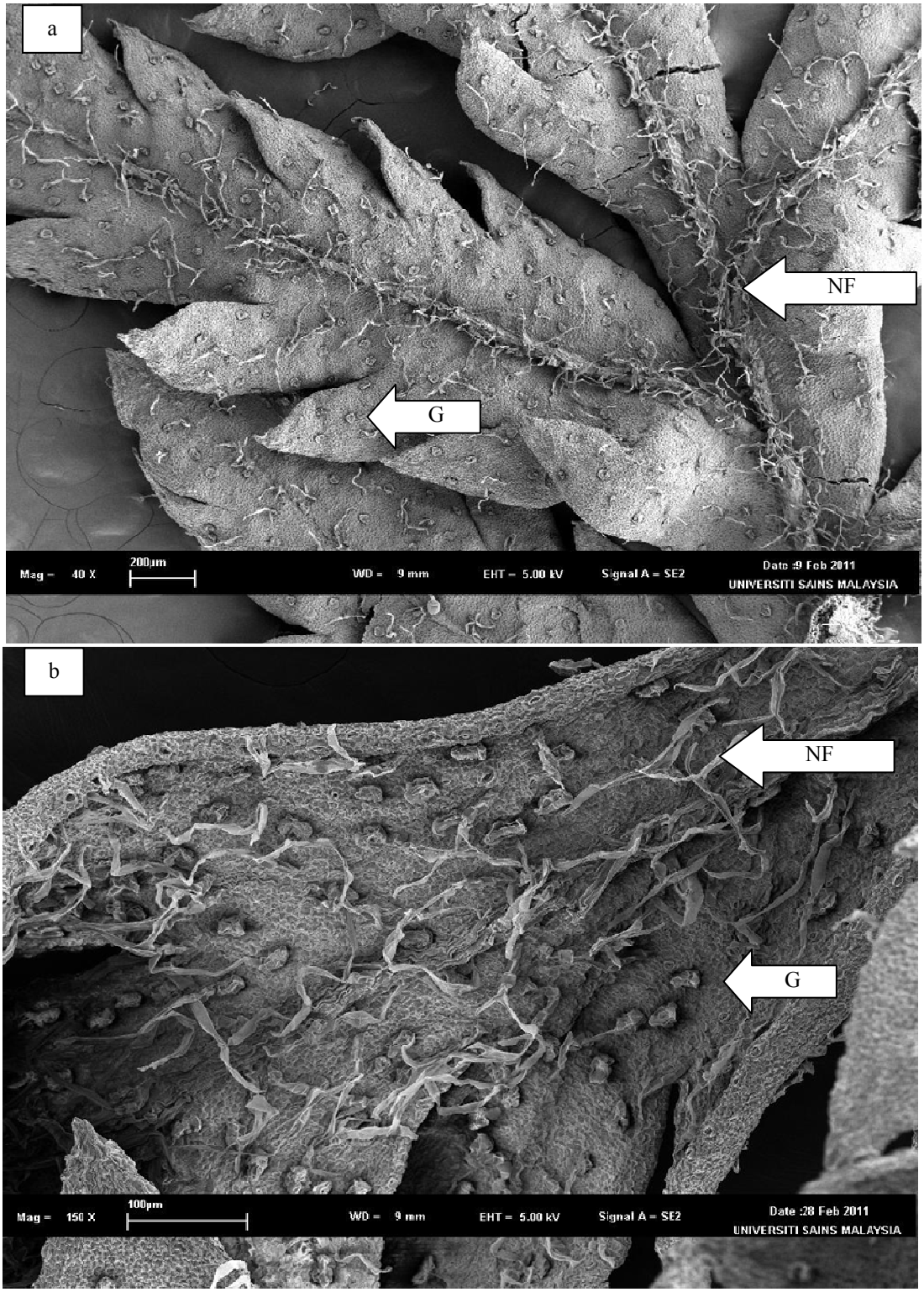


Figure 5. (a) Non-glandular trichomes (NF) in rows along the midrib of abaxial leaf of *A. annua* whereas glandular trichomes (G) were randomly distributed (magnification 40 X). (b) G and NF distributed randomly on adaxial leaf surface adaxial (magnification 150 X).

Clone	MS				B5				WM				LV				NN			
	AD		AB		AD		AB		AD		AB		AD		AB		AD		AB	
	G	NF	G	NF	G	NF	G	NF	G	NF	G	NF	G	NF	G	NF	G	NF	G	NF
TC1	2.5 ± 1 **efgh**	1.2 ± 0 **klmn**	6.7 ± 2 **bc**	4.7 ± 2 **efghi**	3.3 ± 1 **efg**	2.7 ± 1 **fghijklm**	1.0 ±0 **j**	2.3 ± 1 **hijkl**	1.3 ± 1 **ghij**	8.7 ± 2 **cd**	2.7 ± 3 **cdefghij**	8.0 ± 4 **cde**	1.3 ± 1 **ghij**	1.3 ± 1 **jklmn**	11.7 ± 2 **a**	15.0 ± 2 **a**	0.8 ± 0 **j**	1.0 ± 1 **lmn**	4.0 ±1 **cdef**	0.7 ±1 **mn**
TC2	2.0 ± 1 **fghij**	2.0 ± 1 **ijklmn**	2.0 ± 1 **efghij**	2.7 ± 1 **ghij**	1.3 ±0 **j**	4.7 ±0 **e**	3.2 ±1 **ef**	2.3 ±1 **hijkl**	3.3 ±1 **efgh**	3.3 ±1 **efghij**	2.0 ±0 **h**	2.3 ±1 **ijk**	10.0 ±2 **ab**	14.0 ±2 **ab**	6.7 ±2 **bcd**	12.0 ±3 **abc**	1.3 ±1 **ghij**	2.7 ±1 **ghij**	4.0 ±0 **e**	4.7 ±2 **efgh**
High land	1.3 ±0 **ij**	0.8 ±0 **n**	2.7 ±1 **efg**	3.0 ±1 **ghi**	1.3 ±1 **ghij**	4.7 ±1 **ef**	2.8 ±1 **efg**	3.2 ±1 **ghi**	2.7 ±2 **efghij**	2.0 ±1 **ijklmn**	4.0 ±1 **cde**	2.7 ±1 **fghijklm**	3.3 ±2 **defghi**	9.3 ±2 **cd**	8.0 ±1 **b**	4.7 ±1 **efg**	4.0 ±1 **cde**	5.3 ±2 **defgh**	1.3 ±1 **ghij**	4.7 ±3 **defghijk**

Table 1. Effect of basal medium on the number of trichomes on both leaf adaxial and abaxial surface (200 µm x 200 µm area).
G: Glandular trichome; NF: Non-glandular filamentous trichome
Mean values for each type of trichome followed by the same alphabet were not significantly different (Tukey, $p \leq 0.05$).

Table 2. Effect of basal medium on the size (diameter, µm) of glandular trichomes on both leaf adaxial and abaxial surface.
AD: Adaxial; AB: Abaxial.

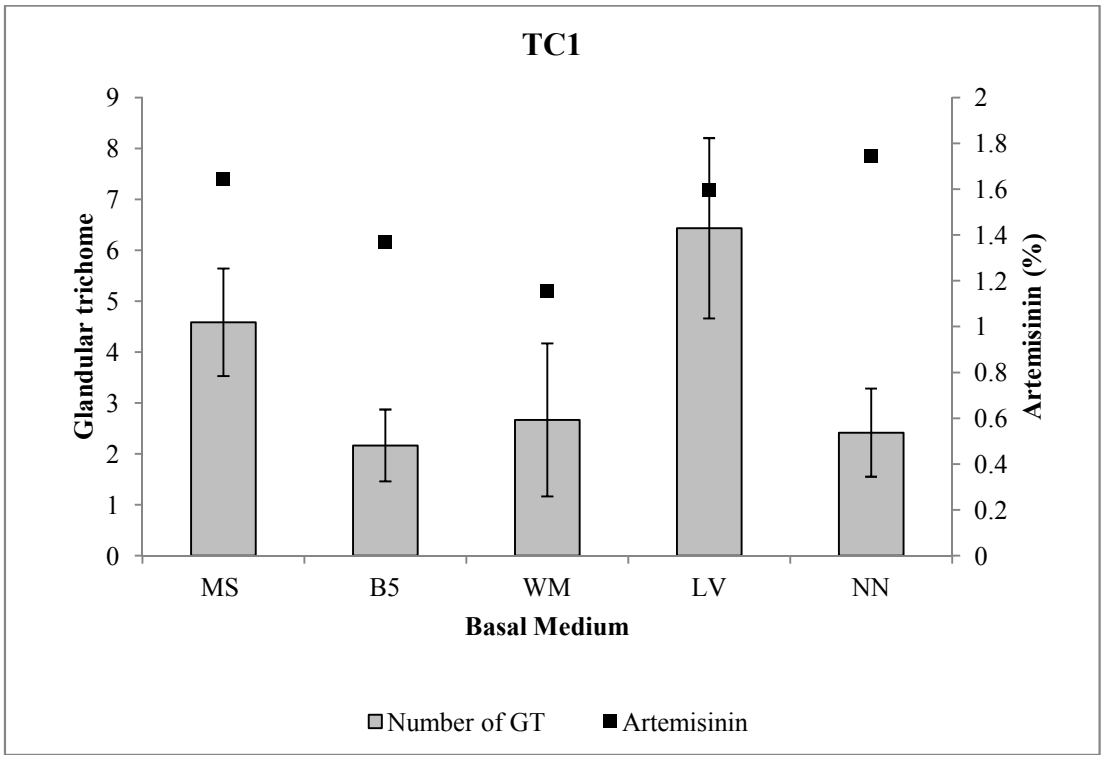
Clone	**MS**		**B5**		**WM**		**LV**		**NN**	
	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB
TC1	33.5 ± 1.9 **ijklm**	32.1 ± 0.9 **mn**	35.8 ± 2.2 **ghijkl**	37.0 ± 1.6 **ghi**	30.9 ± 2.0 **mno**	41.7 ± 1.6 **cde**	27.2 ± 1.3 **pq**	27.0 ± 0.7 **q**	49.2 ± 2.4 **a**	46.7 ± 2.7 **ab**
TC2	32.6 ± 3.1 **ijklmno**	28.7 ± 1.9 **opq**	39.4 ± 1.9 **efg**	35.2 ± 1.8 **hijkl**	39.5 ± 2.5 **defg**	40.4 ± 1.5 **def**	29.1 ± 1.3 **op**	34.7 ± 1.0 **ijkl**	37.2 ± 1.5 **gh**	35.2 ± 1.1 **hijk**
Highland	39.0 ± 1.7 **efg**	34.4 ± 2.3 **hijklm**	43.2 ± 1.7 **bcd**	40.6 ± 1.7 **cdef**	21.0 ± 1.2 **r**	38.8 ± 2.5 **efgh**	30.5 ± 0.9 **no**	33.0 ± 1.1 **lm**	36.1 ± 1.6 **ghij**	45.6 ± 3.3 **abc**

Mean values of the length of glandular trichomes for each clone in different basal medium followed by the same alphabet were not significantly different (Tukey, $p \leq 0.05$).

Table 3. Effect of basal medium on number of stomata on both adaxial and abaxial (± standard error) of the leaf surface (200 µm x 200 µm area).
AD: Adaxial; AB: Abaxial

Clone	MS		B5		WM		LV		NN	
	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB
TC1	5.3 ± 2.0 **gh**	19.3 ± 3.6 **bc**	19.3 ± 1.9 **c**	0.0 ± 0.0 **k**	16.7 ± 4.2 **cde**	19.0 ± 8.3 **bcdef**	15.3 ± 3.0 **cde**	8.0 ± 3.6 **fgh**	1.7 ± 1.0 **hi**	4.0 ± 1.5 **ij**
TC2	28.0 ± 5.2 **b**	40.0 ± 5.8 **a**	5.0 ± 1.2 **gh**	0.0 ± 0.0 **k**	7.3 ± 1.2 **g**	14.7 ± 3.8 **cdef**	1.7 ± 1.3 **ij**	1.7 ± 0.8 **ij**	0.0 ± 0.0 **k**	22.0 ± 4.1 **bc**
Highland	20.0 ± 3.1 **bc**	11.5 ± 1.4 **ef**	20.7 ± 2.6 **bc**	0.0 ± 0.0 **k**	18.0 ± 4.8 **bcd**	0.0 ± 0.0 **k**	3.3 ± 2.6 **hij**	16.7 ± 3.6 **cd**	0.7 ± 0.7 **jk**	12.0 ± 3.4 **def**

Mean values for number of stomata for each clone in different basal medium followed by the same alphabet were not significantly different (Tukey, $p \leq 0.05$).



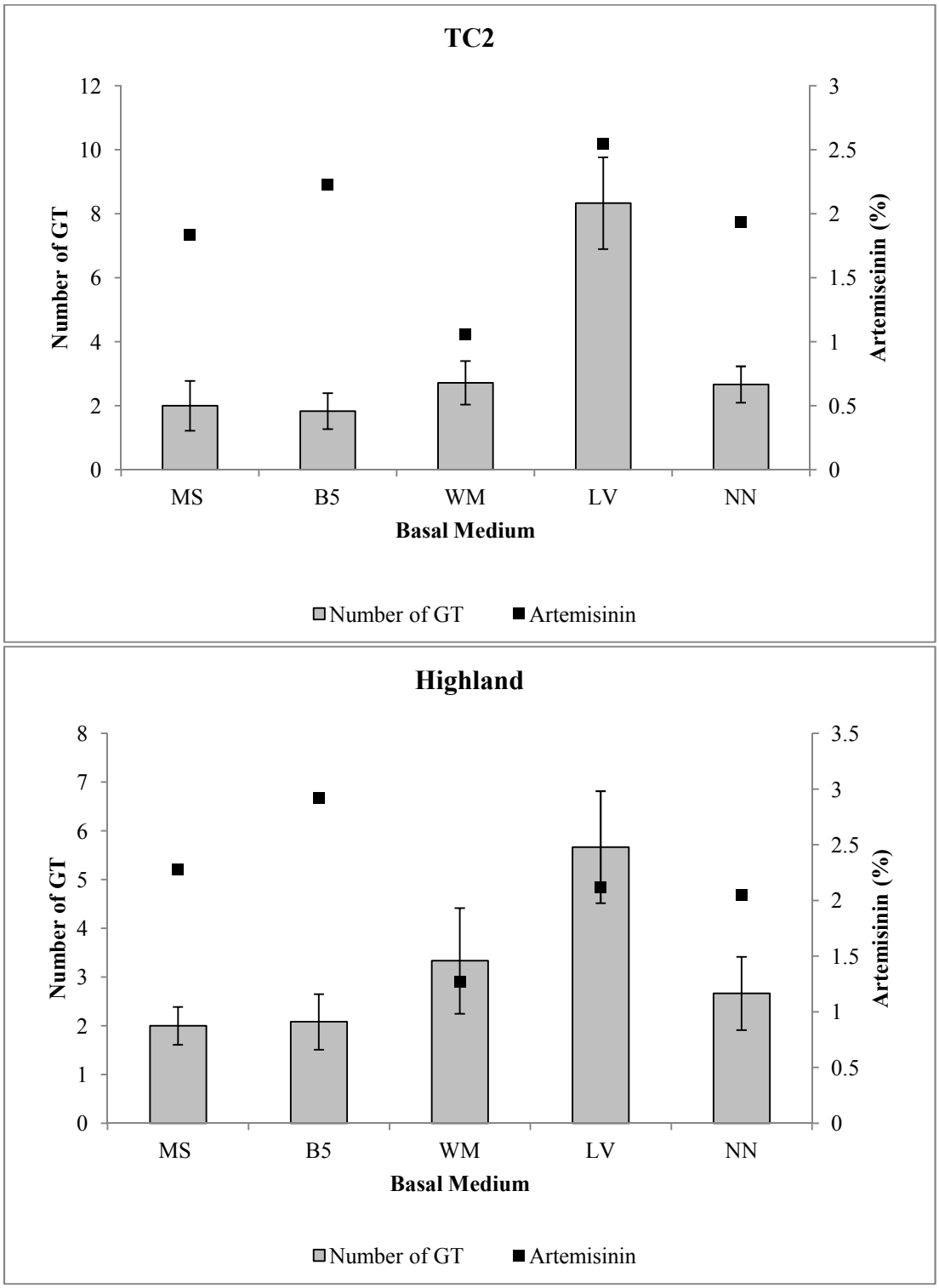


Figure 6. Correlation between the total numbers of glandular trichome (GT) on leaves (adaxial and abaxial, 200 x 200 μm^2) and content of artemisinin (% of the dry weight).