Validating Field Regeneration Capacity for Selected Accessions of *Gossypium hirsutum* Using Callus Induction and Regeneration Capacity

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Abstract

Gossypium hirsutm undergoes a rapid clonal propagation to regenerate a mature plant through tissue culture. In this research, cotton leaf regeneration level for 21 accessions in the field (new leaves) was observed after the first harvest, and a comparison between field regeneration level and callus induction with its regeneration capacity (new shoots and roots) for the same 21 accessions was carried out. During the flowering stage of Gossypium hirsutum, biochemical (Proline), physiological (chlorophyll and carotenoid content) analysis was carried out. Phenotypic observations (plant height, leaf area, fresh leaf weight, dry leaf weight, flower and boll number) were also carried out on 21 accessions for each accession. Callus induction and regeneration capacity of roots and shoots for hypocotyl, cotyledons and shoot tip tissues was used to validate field regeneration capacity through analysis of variance. ZS061, LuMian378, JiMian863, and ZS065 have highest drought tolerance while ZhongMianSuo24, LiaoYangDuoMaoMian, and BeiZheGongSheMian have the lowest tolerance to drought stress. Accessions with both field and callus regeneration capacity were identified.

Introduction

Cotton (*Gossypium spp.*) is a widely grown textile crop worldwide. It has 52 species that can be found all over the world, mostly in subtropical and tropical areas. Cotton is also an important oilseed crop globally of high economic value (Chen et al. 2007). Upland cotton (*Gossypium hirsutum*) is widely grown in over 80 countries, covering almost 33 million hectares, or roughly 5% of the world's total arable (Dai et al. 2015; Yu et al. 2016). During the growing season, cotton plants shed their leaves in response to drought, disease, nutrient imbalance, frost, or other environmental stress. However, when the conditions improve, new leaves tend to regenerate. This is a sign of cotton tolerance to new environmental conditions and the ability to adapt (Chawla et al. 2012). Regeneration is the ability of multicellular organisms to regenerate or grow new cells, tissues, or even whole organs in response to injury or wounding (Xu and Huang 2014). Every species has unique regenerative abilities, and each organ within a single organism can uniquely respond to regeneration (Oki and Kanae 2006). Tropical cotton crops can have diverse reactions to insect damage and appear to recover more quickly when subjected to some environmental stress (Yeates et al. 2010).

Leaf shedding is caused by the activity of specific cells near the base of the leaf petiole, where it connects the stem (abscission). This region is referred to as the "abscission layer." Defoliation can be caused by a mild frost, insect devastation, illness, drought, or mineral shortage, as well as the use of chemicals known as "defoliants" or harvest aids. Also after the first harvest or a drop in temperature, some cotton varieties regenerate new leaves (Tian et al. 2015). During tissue culture, adventitious roots or shoots can be generated by shifting the callus to a media containing varying ratios of auxin and cytokinin. De novo organogenesis, or the synthesis of a new plant from separated organs, can often contribute to the establishment of a new plant at a wound site without the necessity for callus formation. *Arabidopsis thaliana* is a model plant that has been used to explore de novo organogenesis (Liu et al. 2014). Early-season variables such as cold, wet weather, soil crusting, seedling disease, and hail or wind damage can all hamper cotton germination and emergence. Furthermore, little is known about the ability of damaged seedlings to recover after partial or complete leaf loss (Longer and Oosterhuis 1999). It was revealed that the regeneration capability of older cotton plants reduced as the severity of injury rose or as the same damage was applied to older cotton plants (Butts et al. 2019). This ability to direct the fate of differentiated somatic cells aids tissue repair and organ reconstruction following an injury during postembryonic development, as well as de novo formation of various plant structures in *Gossypium hirsutum*, from in vitro explant cultures in response to phytohormones or abiotic stresses, a process known as regeneration, which has a variety of biotechnological applications. To validate field regeneration potential of *Gossypium hirsutm*, tissue culture studies are required, and efficient rooting is just as important as achieving the highest shoot induction and regeneration response. Using in vitro culture of different tissues, various cotton cultivars have been investigated for direct or indirect regeneration and callus induction. In upland cotton (*Gossypium hirsutum*), the solid-liquid alternating culture method could speed up embryogenesis through callus induction and reduce the time it takes for upland cotton plants to regenerate new tissues (Liu and Wang 2020).

Numerous studies have clearly induced various explants into calluses, including hypocotyl, cotyledon, root, leaf fragments, immature embryo, and nodal explants in upland cotton (Surgun 2014). In addition, some studies reported shoot regeneration in few species (Cheruvathur et al. 2010; Mungole et al. 2011; Subban et al. 2021 Morre et al. 1998). It was reported that at the molecular level, some transcription factors, such as BABY BOOM (BBM), play a significant role in the signal transduction pathway and resulted in cell differentiation and the formation of somatic embryos and leads to the formation of more new cells and tissue regeneration when apical meristem, shoot apex, and cotyledon nodes have been used as explants (Loyola-vargas et al. 2019). Many studies have revealed the mechanisms of plant regeneration ability at the molecular level in upland cotton, but evaluation of regeneration capacity in cotton at the callus level has still not been fully elucidated. A callus regeneration capacity of cotton plants was used to validate the actual field regeneration capacity of cotton in this study.

Material And Methods

Seeds selection, planting, and germination

Seeds of 21 upland cotton accessions were received from the Mid-term gene bank in Cotton Research Institute of the Chinese Academy of Agricultural Sciences. On May 25, 2020, the seeds were planted in the field. The planting row is 7 m and each plant is spaced 30-40 cm apart. Three replicates were used in the field experiments, which were set up in a randomized full block configuration (Juan and Yuqiang 2015). We investigated the number of germinated plants after sowing two weeks and determined the germination percentage of each accession as follows:

$$\mathrm{GP} = \frac{\mathrm{n}}{\mathrm{N}}*100$$

Where, N: Total number planted in the field, n: Number of germinated plants and GP: germination percentage

Phenotypic observations

For each of the 21 accessions, number of flowers and bolls, leaf area, plant height, fruit branch number, and leaf branch number were recorded one by one. For each trait, we have observed 20 plants. Plant height was measured by using a 150 cm meter ruler, leaf area was measured using a Vernier caliper, and the fresh weight of each leaf in all accessions was recorded using a weighing balance.

Field regeneration level investigation

Newly growing leaves were counted and recorded after harvesting. The field regeneration capacity was represented as follows;

Where, No new leaf produce: 0, One new leaf produce: 1, Two new leaves produce: 2 and Three or more new leaves produce: 3

The regeneration level was carried out by direct counting one by one for 20 plants and their respective values for all the above categories were recorded.

Moisture contents determination

A mature leaf was removed from 20 plants for each of the 21 accessions during the flowering stage. After we recorded the leaf fresh weight (WF), leaves were air dry for two days and recorded the leaf dry weight (WD) and the net weight lost (WN) was calculated as WN WF—WD.

Relative electronic conductivity analysis

Leaf of 1 cm diameter was cut from fully matured and expanded leaves from 20 plants for each accession and washed three times with de-ionized water and then placed in test tubes with 10 mL de-ionized water. The test tubes were placed at room temperature for 24 hours before the EC of the solution (T1) was measured using a calibrated EC meter. Then the test tubes were capped and autoclaved for 15 minutes at 121°C. When the solution had cooled to 20°C, the EC was measured again and recorded as T2, and the relative electronic conductivity was calculated as T1/T2 (Cottee et al., 2010). The experiment was carried out three times.

Estimation of total carotenoid content

The leaves from 20 plant for each accession were cut into small pieces which about 0.5 g and grind with a mortar and pestle with 10 ml of (80%), the extract was centrifuged at 2500 rpm for 10 minutes and made up to 10 ml with 80% distilled acetone. The absorbance of the extract was read at 480 nm and 510 nm. The total amount of carotenoids (C) was calculated by using the following formula:

$$C = (7.6 \times OD\ 480) - (1.49 \times OD\ 510) \times V \times W$$

Where V = Final volume of supernatant; W = Weight of the leaf sample taken in gram.

All the analyses were carried out in three replicates for each plant (Zaghdoudi et al. 2015).

Estimation of chlorophyll content and total proline content

SPAD-502 meter was used to measure the chlorophyll content on the matured leaf on three portions on three different leaves of each plant. 20 plants of each accession have been measured. (León et al. 2007). Proline content also extracted and estimated by using a cold extraction procedure (Manuscript 2010).

Callus induction and tissues regeneration capacity

Cotton seeds from all accessions were delinted and sterilized with 0.1% $HgCl_2$, which was shaken for eight minutes before being rinsed five times with distilled water in 2:2:1 minute's rations. The sterilized seeds were placed into a plate and put into a dark growth chamber which set at 28°C for 48 hours. Then the young germinating seedling was transplanted into the bottles and placed in a dark chamber set at 28°C for five days. Seedlings were cut into hypocotyl, cotyledon, and shoot tip at seven days old. Hypocotyl and cotyledon were transplanted into MS media containing 2,4-D (0.1 mg/mL) and kinetin (0.1 mg/mL), respectively, and shoot tips were transplanted into MS medium containing 2,4-D (0.2 mg/mL) and kinetin (0.2 mg/mL). For 14 days, all tissues were placed in a light chamber (16 hours of light and 8 hours of darkness) at 28°C, and the callus induction rate was calculated as follows:

2 x WCIR = $\delta y/\delta x \times 100$

2 WCIR: Two weeks' callus induction rate

δy: Total number of callus tissue formed within two weeks per plate

δx: Total number of tissue in plate within two weeks

Two weeks later all the tissues were switched to new MS medium with the same phytohormone supplement and growth for another two weeks, during which time the callus induction rate was monitored and documented once more. Plates with good callus induction were chosen and left to see if shoots and roots from three different tissues might regenerate.

$$4WCIR = \frac{\delta y}{\delta x} \times 100$$

4 x WCIR: Four weeks' callus induction rate

δy: Total number of callus tissue formed within four weeks per plate

δx: Total number of tissue in plate within four weeks

Results

1. Agronomic trait analysis

In the field investigation after successful planting and germination with control of other factors necessary for growth and development such as pest and drought control through pesticide and irrigation, the plant produced efficient leaves, fruits, flowers, and bolls. Plant height (PH), leaf branch number (LBN), fruit branch number (FBN), number of flower and boll (NFB), leaf area (LA) and leaf weight lost (LWL)were measured and compares for all lines (Fig. 1).

Plant height

The Plant height (PH) of all 21 accessions have significant variation (from 51.65 cm to 99.85 cm) according to an analysis of variance with p-value (p 0.01). The highest accession was LuMian378 and the lowest accession was JunMian1Hao (Fig. 1A).

Fruit branch number

Fruit branch number was an important trait for fiber yield in cotton. Five accessions Ari971, Si-6524, B557, Dai15, and Bole34 have more fruit branch number (from 13.05 to 13.74) than the other three accession ZS065, SuJiMian211 and ZS061 (9.7, 9.3 and 9.05, respectively, Fig. 1B).

Number of flower and boll

The flower and boll number comparison result revealed that five accessions Bole34, Ari971, Dai15, ESha218hao and DES926 have the highest flower and boll number (from 15.35 to 18.10). However, FH682 exhibit lowest flower and boll number (3.95) accordingly (Fig. 1C).

Leaf branch number

Leaf branch number (LBN) was a significant trait for cotton yield and 21 accessions have a significant difference in LBN according to the analysis of variance at p 0.01. Ari971 and Si-6524 have the highest LBN (13.55 and 12.45, respectively). Those accessions with lowest LBN was JunMian1hao, BeiZheGongSheMian, LiaoYangDuoMaoMian, Dai15, ZS061 and ESha218hao (from 4.55 to 9.3, Fig. 1D).

Leaf area and leaf weight lost

Leaf area (LA) has a direct impact on the photosynthetic rate of cotton plants. According to the variance analysis result, ZS061, LuMian378, JiMian863, and ZS065 have the highest LA and LWL values, while JunMian1Hao, ZhongMianSuo24, LiaoYangDuoMaoMian, and BeiZheGongSheMian have the lowest LA and LWL values. These result indicated that cotton plants with bigger leaf will lost more weight after drought stress.

2. Biochemical analysis

Photo-inhibition occurs when high temperatures and drought degrade cell membrane structure, cause proline synthesis, and reduce chlorophyll concentration, resulting in leaf senescence (A et al. 2016). Ion leakage is a crucial element in determining cell membrane stability and can be used in the assessment of abiotic stress tolerance like water stress and heat stress. Our result showed that three accessions Bole34, N98-283 and FH682 (from 4.95–5.65%) were more tolerant than other accessions especially JiMian863 (22.24%) and LuMian319 (21.32%) (Fig. 2A).

Chlorophyll and carotenoid content were correlated to the photosynthetic potential of plants and give some indication of the physiological status of the plants (Gamon & Surfus, 1999). In our result, the chlorophyll of SuJiMian211 and 2019Y-3 were significantly high than ZaoYang BuLuoLei, B557, and JumMian1Hao. However, the changing trend of the carotenoid content in all 21 accessions were different. N98-283, ZS061, Si-6524, and ZhongMianSuo24 were significantly high than other accessions (Fig. 2B-C).

Proline content was another important parameter for abiotic stress tolerance. Our result showed that ZS061, ZhongMianSuo24, N98-283 and Si-6524 showed higher proline content than the rest of the other accessions (Fig. 2D).

3. Regeneration capacity analysis by field investigation

Some lines of upland cotton growth new leaves after harvest when the temperature drops and old leaves tend to fall away. These emergences of new leaves have been categorized into three levels (Fig. 3). The first category is plenty of new leaves produce (3) which have three new leaves and above, the medium level (2) which have two new leaves, level 1 which have only one new leaf, and level 0 means no new leaf growth. Each of these four levels was observed and assessed for all the 21 accessions and from the result we can know than first four accessions (B557, LuMian378, JiMian863, and LuMian319) have high regeneration rate, three accessions (BeiZheGongSheMian, LiaoYangDuoMaoMian, and ZhongMianSuo24) have low regeneration rate, while other accessions have medium regeneration rate (Fig. 4).

4. Callus induction and regeneration capacity analysis

Callus induction was carried out for 21 accessions with three different tissues (hypocotyl, cotyledon, and shoot tip) and their respective callus induction rate and regeneration capacity (shoot and root regeneration) were analyzed and recorded.

The first clade which including LuMian319, ZA065, LuMian378, and 2019Y-3 have high hypocotyl and cotyledon callus induction rate but have low callus induction rate. The second clade which including six accession showed both high callus induction rate in three tissues but the level has some difference. ESha218Hao and SuJiMian211 have little low callus induction rate in shoot tip than hypocotyl and cotyledon, Ari971 and ZS061 have little low callus induction rate in cotyledon than hypocotyl and shoot tip. For B557 and Bole34, they have significant high callus induction rate in both three tissues. The third clade which including four accession (JunMian1Hao, ZaoYangBuLuoLei, ZhongMianSuo24, and DES926) showed significant low callus induction rate in hypocotyls and cotyledon than shoot tip. The forth clade is JiMian863 which have medium callus induction rate in both three tissues. The last clade including five accessions which have little high callus induction rate in shoot tip than hypocotyl and cotyledon. From these result, we can know that one accession has different callus induction rate in different tissues and we can use those accessions in clade 2 and 5 to do the next embryogenic callus induction experiment and so on (Fig. 5A).

During callus induction period, some tissues have shoot and root growth (Fig. 5B). For hypocotyl tissue, only one accession ZS061 regenerated new root, while twelve accessions (LuMian378, ZaoYangBuLuoLei, DES926, ZS061, ZhongMianSuo24, LuMian319, 2019Y-3, ZS065, Ari971, Esha218Hao, Si-6524, and N98-283) were observed to have new shoot regeneration in the hypocotyl. In cotyledons, eleven accessions (ZS061, ZhongMianSuo24, LuMian319, 2019Y-3, ZS065, Ari971, Esha218Hao, Si-6524, N98-283, Bole43, LiaoYangDuoMaoMian) were observed to have root generation but only one accession (Esha218Hao) has shoot regeneration. Three accessions (B557, Si-6524, N98-283) have root regeneration and eleven accessions (B557, FH682, Dai15, BeiZheGengSheMian, JunMian1Hao, Ari971, Esha218Hao, Si-6524, N98-283, Bole34, LiaoYangDuoMaoMian) have shoot regeneration in shoot tip tissue. For accessions, SuJiMian211 and JiMan863 didn't growth shoot or root in all three tissues. From this result, we can have known than hypocotyls and shoot tip tissues tend to generate shoot during callus induction period, while cotyledon tend to generate root during callus induction period and the regeneration capacity for different accessions showed some difference.

Discussion

In this study, we carried out phenotypic, physiological, biochemical, analysis, and field regeneration capacity for 21 accessions of the upland cotton and also used callus induction of the same 21 accessions on hypocotyl, cotyledons, and shoot tip tissues to check the regeneration of shoots and roots from these three tissues to validate the field regeneration capacity of each accession. The height of cotton plants is a result of active cell division and favorable weather conditions for appropriate growth and development. Plant height gives a good affinity to light intensity and in turn proper photosynthesis. According to our finding, three accessions (JiMian863, LuMian378, and LuMian319) were discovered to have the highest plant height than other accessions. Light is a source of energy as well as an important signal for environmental changes, causing a variety of physiological reactions in plants (Abidi et al. 2013). According to the previous report it was revealed that photoperiod significantly influenced photosynthesis, seed germination, breaking of dormancy, and the flowering process (Skjelva 2004; Rezazadeh and Harkess 2018). Our finding revealed that there is a significant correlation between plant height and leaf area on ZS065 accession, this trait (height) has a significant role in the growth and development of upland cotton due to large leaf area to trap enormous sunlight, It was reported that plant height is an important selection target since it is associated with yield potential, stability, and particularly with lodging resistance in various environments (Hassan et al. 2019). Flowering onset time has been reported to be positively correlated with maximum plant height (Hmax). Herbaceous grassland species, taller species often flower later than shorter ones (Dahlgren et al., 2007). On the JiMian863 accession, we discovered a significant relationship between leaf area and plant height. In the JiMian863 accession, there is new shoot regeneration in callus induction.

Low leaf area and leaf branches were observed on JunMian1Hao accession, which corresponds to a high level of carotenoid. This could be due to the low amount of sunlight strike the surface of leaves. The concentration of photosynthetically active pigments increases in carotenoid content under the shade condition and this is applied to chlorophylls and carotenoids in green algae (Czeczuga 1987). There is a significant correlation between shoot tip callus induction and shoot regeneration capacity on JunMian1Hao. Low moisture content and low conductivity were observed on JunMian1Hao accession. The high percentage of re-generable embryogenic calli is a prerequisite for genetic manipulation towards varietal improvement. This study aimed to test and improve the embryogenic potential of calli for better plant regeneration efficiency (Juturu et al. 2016). Flower and boll number is an important trait for upland cotton. A large number of boll produces enormous fiber contents. Three accessions were found to have the highest number of flowers and bolls (Bole34 and Dai15) in comparison to new tissue regeneration on these accession new shoot and root were observed in cotyledon and shoot tip tissues. This is an indication that good callus induction induces new tissues regeneration in shoot tip and cotyledon tissues of Bole34 and Dai15 accessions.

Moderate callus induction was observed on LuMian319 accession for shoot tip tissue with a significant correlation for shoot regeneration. For the same Bole34 accession, there was the highest shoot tip callus induction (100%), which correlates with shoot regeneration, similar findings for root regeneration of cotyledon tissue similarly hypocotyl and shoot tip tissues produce a new shoot tissue. Multiple shoot and root regeneration correlates with the maximum callus induction capacity. In the field, three accessions were also discovered to have limited or low flower and boll (JunMian1Hao and FH682) with no new leaf produce in some accessions. Callus induction capacity was used to confirm the new tissues regeneration levels on these accessions. Shoot tip callus induction with corresponding shoot regeneration was observed on JunMian1Hao accessions. Low leaf branches, low moisture content, and low conductivity were observed on JunMian1Hao accession, this may be attributed to the effects of moisture on conductivity level. Thermal conductivity depends on temperature and moisture content. In comparison shoot, tip callus induction (100%) correlates with good shoot regeneration capacity on FH682. In hypocotyl callus induction high callus (100%) response was observed on nine accessions which also correlates with shoot regeneration in 12 accessions. There is a similarity between callus induction and new tissue (shoots and roots) regeneration observed in hypocotyl in seven accessions (ZS061, Ari971, Esha218Hao, 2019Y-3, LuMian378, ZS065, and LuMian319). It was also discovered that accessions with low flower and boll numbers in the field correlate with a low callus induction level and low regeneration capacity in hypocotyl tissue (JunMian1Hao and ZhongMianSuo24), the same accession also displays a limited response to regeneration capacity. Our finding also revealed that callus induction abilities are greatly influenced by the genotype and are in agreement with the previously reported work in Oryza sativa (Abe and Futsuhara 1986). Culture medium composition, genotype, and its condition are important factors affecting callus induction and its regeneration rate. Among those factors, the genotype appears to be a more important factor affecting the efficiency of in vitro culture. In *Triticum aestivum*, the explants with the same age and the same growth regulator combination, callus production and plant regeneration capacity depend essentially on genotype (Arzani and Mirodjagh 1999). Fruit branches is an important trait for upland cotton, the quality of cotton fiber results from an interaction between environmental, genetic, and management factors, determined by the position of the resources obtained by each fruit (Percy et al. 2006; Girma et al. 2007).

Cotton morphology is primarily determined based on flowering and shoot branching patterns which directly influence sunlight distribution, yield, planting area, the efficiency of harvest mechanization, and the cost of planting (Reinhardt and Kuhlemeier 2002; Sakamoto and Matsuoka 2004). The net weight of each leaf of the 21 accessions was weighed and determined. the water retention capacity, which was used to determine the relative amount of water which indicates drought resistance levels of each accession. According to the mean comparison values, two accessions (ZS065 and ZS061) have the strongest affinity for drought resistance than the rest of the accessions. Similarly, the lowest affinity to drought resistance was observed on (BeiZheGongSheMian and LiaoYangDuoMaoMian) according to mean comparison values. In LuMian319 accession, a significant correlation was observed for both moisture content and net conductivity. This is possibly due to genetic variation in the genome constituent within the *Gossypium hirsutum*. It was reported that plants are evolved to regulate growth periods to avoid moisture stress, termed as drought escape (Manavalan et al. 2009). The first response of plants to drought as a drought-resistance strategy relies on avoiding water deficit (Zonta et al. 2017). by maintaining tissue weight through increasing water uptake or restricting water loss (Antunes et al. 2018). Leaf conductivity was asses for membrane stability on 2019Y-3 accession and it was found out that it correlated with higher chlorophyll content and it’s also similar a related finding that photosynthetic stimulation was correlated with increased leaf conductance (Chen 2000).

Carotenoid content which functions in trapping the sunlight for photosynthesis, the values of each accession were measured and recorded. There was a significant correlation on the value of carotenoid in comparison to other traits. On rare finding high carotenoid content correlated with high values of chlorophyll content on (N98-283, ZS061 and Si-6524) accessions respectively. Cotton which attained a required height doesn’t necessarily need high carotenoid content to trap more sunlight to attain maximum growth. High carotenoid content was recorded on N98-283 accession (2.0377) with low cotton height, in comparison to other accessions with the low value of carotenoid so high carotenoid content doesn’t necessarily correlate with good height and this can be supported on view to the intensity of plant height which increased at low light intensity (Wang and Li 2008). Proline is an important trait in Gossypium hirsutum which overcome biotic and abiotic stresses. In comparison for Proline levels among 21 accessions, it indicated that JunMian1Hao accession has the highest Proline content and low-moderate moisture content and this support the finding that plants were subjected to a drying cycle when the plants have four true leaves and the Proline content of the leaf tissue was determined (Chen 1966). In callus induction and new tissue regeneration, there are so many correlations among the 21 accessions. Nine accessions have 100% callus induction capacity for hypocotyl tissue in comparison to the regeneration capacity and callus induction on hypocotyl tissue, no callus induction formed below 50% was observed in all the three tissues (hypocotyl, cotyledons and shoot tip). The highest callus induction was observed in shoot tip tissue and this is possibly due to the presence of meristematic cells at the tip end of the seedling in Gossypium hirsutum. It was reported that high percentage of re-generable embryogenic calli is a prerequisite for genetic manipulation towards varietal improvement (Juturu et al., 2016). Embryogenic callus is best requirement for successful tissues regeneration. Successful callus induction and subsequent tissues regeneration depends on exogenous supply of plant hormones (Rueb et al. 1994; Samota et al. 2017)

Conclusion

The field regeneration capacity was examined, and there was a variation in regeneration levels across the 21 accessions. In all 21 accessions, we found a substantial relationship between field regeneration capability and callus induction with its regeneration capacity for the hypocotyl, cotyledons, and shoot tip. There was also a significant variation among different traits observed. Accessions with similarity on regeneration capacity in both fields and tissue culture, more work needs to be done on other accessions so that GWAS analysis can be carried out to find out the genes related to their respective regeneration capacity and other related traits.

Declarations

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Authors' contributions

S.M.T and X.D conducted the experiment, S.M.T wrote the manuscript, X.G and M.T helped in data analysis. B.C, D.H, L.W, S.B.S, ZP, S.K, M.S. SI helped in manuscript revision.

Statements of Declarations

No competing interest as declared by all authors. I also declared that all the data used in the manuscript are original generated by myself and my laboratory colleagues in both field and laboratory.

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Figures

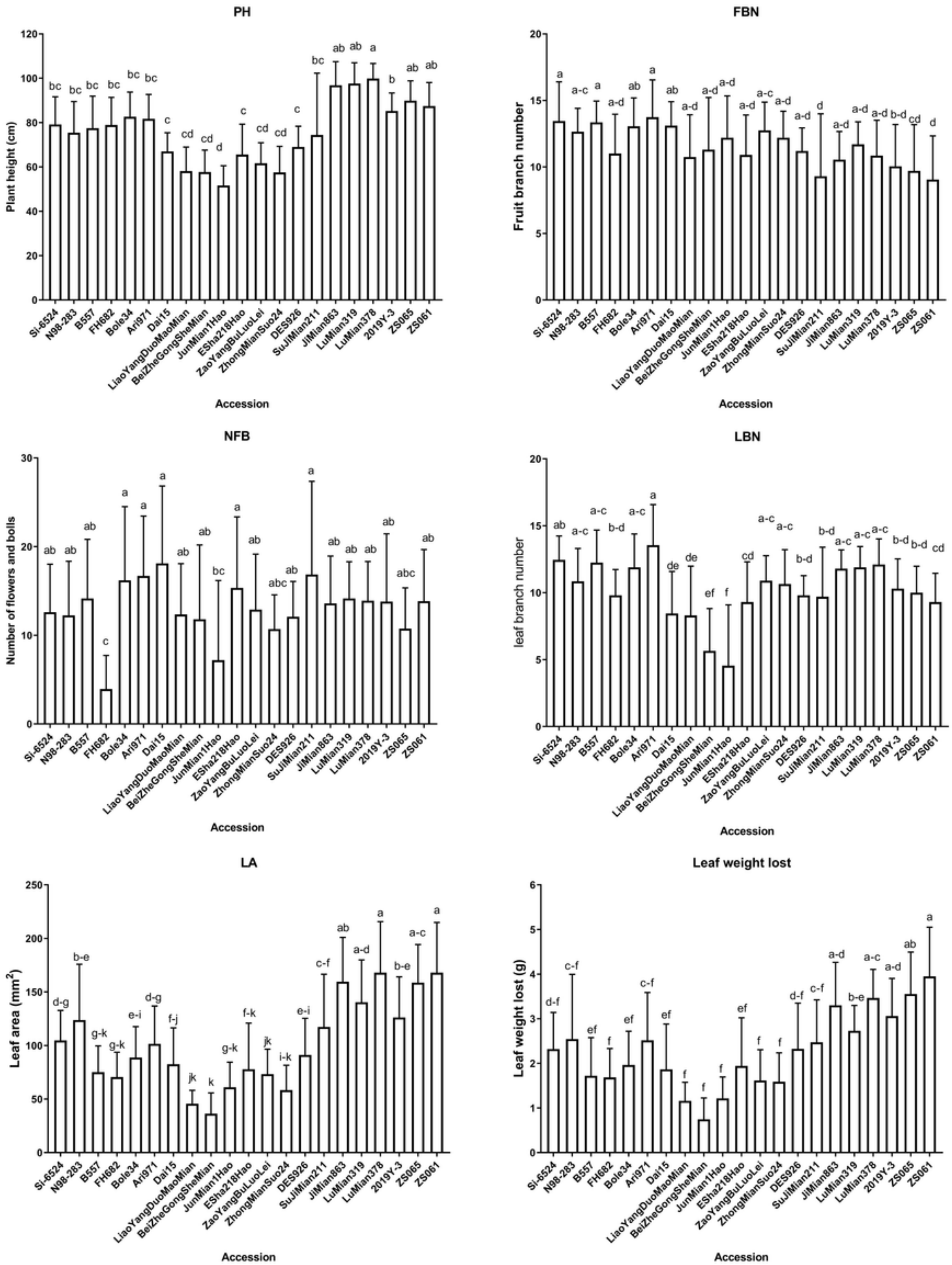

Figure 1

Variation analysis on four agronomic traits in 21 accessions. Bars with different letters indicate a significant difference ($p < 0.05$) using one way-ANOVA. PH (Plant height), FBN (Fruit branch number), NFB (Flower and boll number), LBN (Leaf branch number), LA (Leaf area)

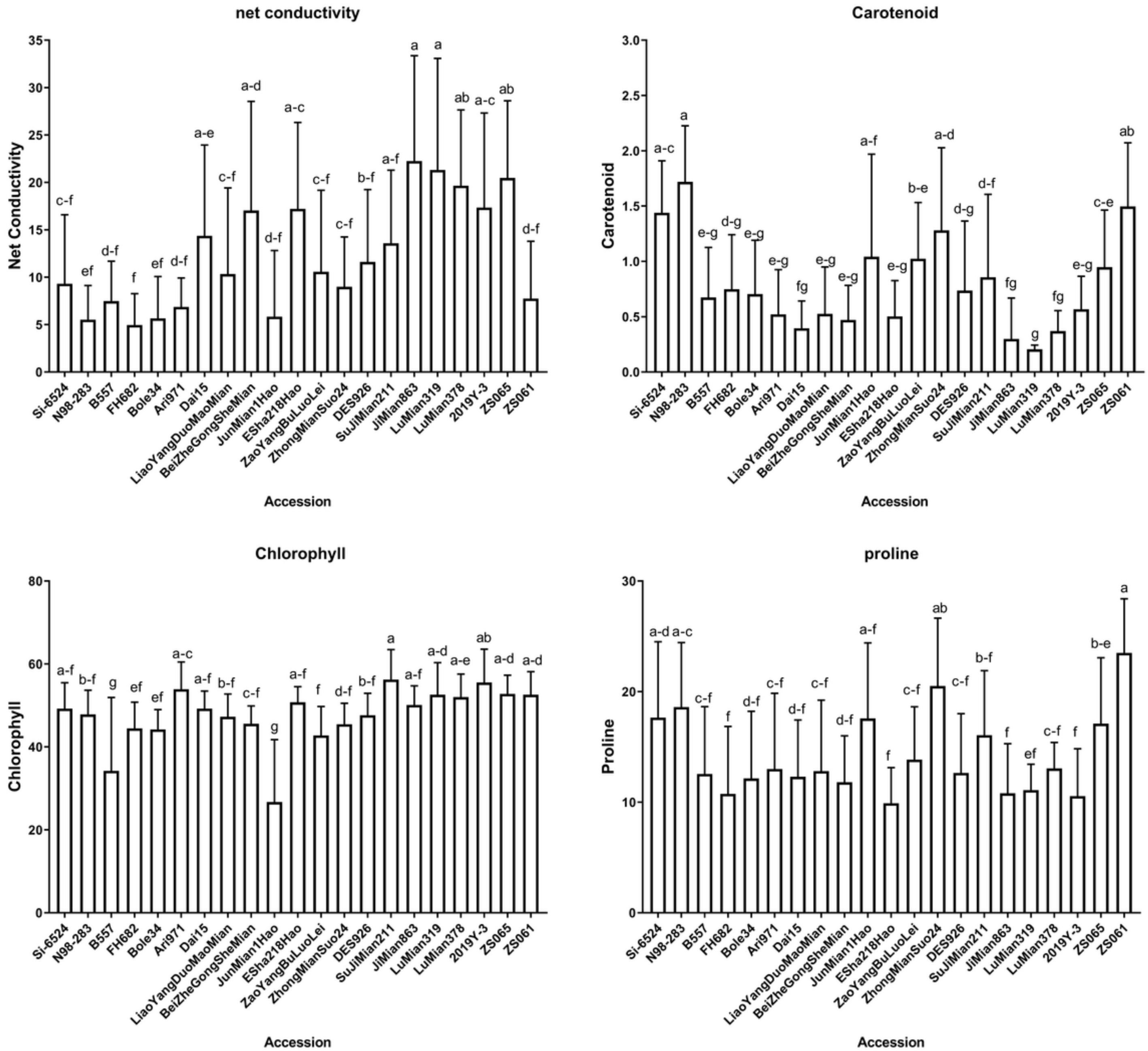

Figure 2

Variation analysis on biochemical traits for 21 accessions. Bars with different letters indicate a significant difference ($p < 0.05$) using one way-ANOVA.

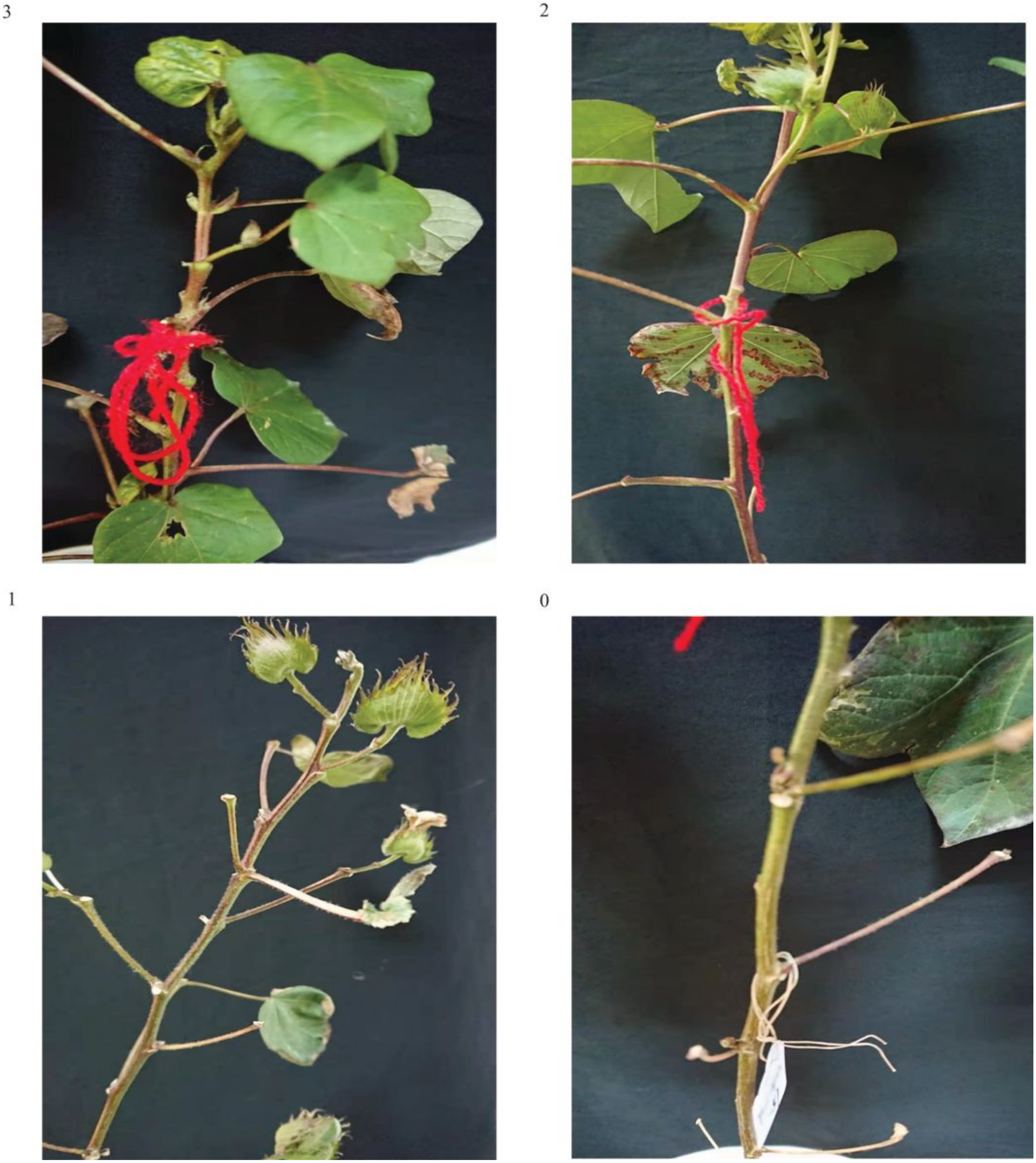

Figure 3

Different field regeneration levels. 3: more than three new leaves produced; 2: two new leaves produced; 1: one new leaf produced; 0: no new leaves produced.

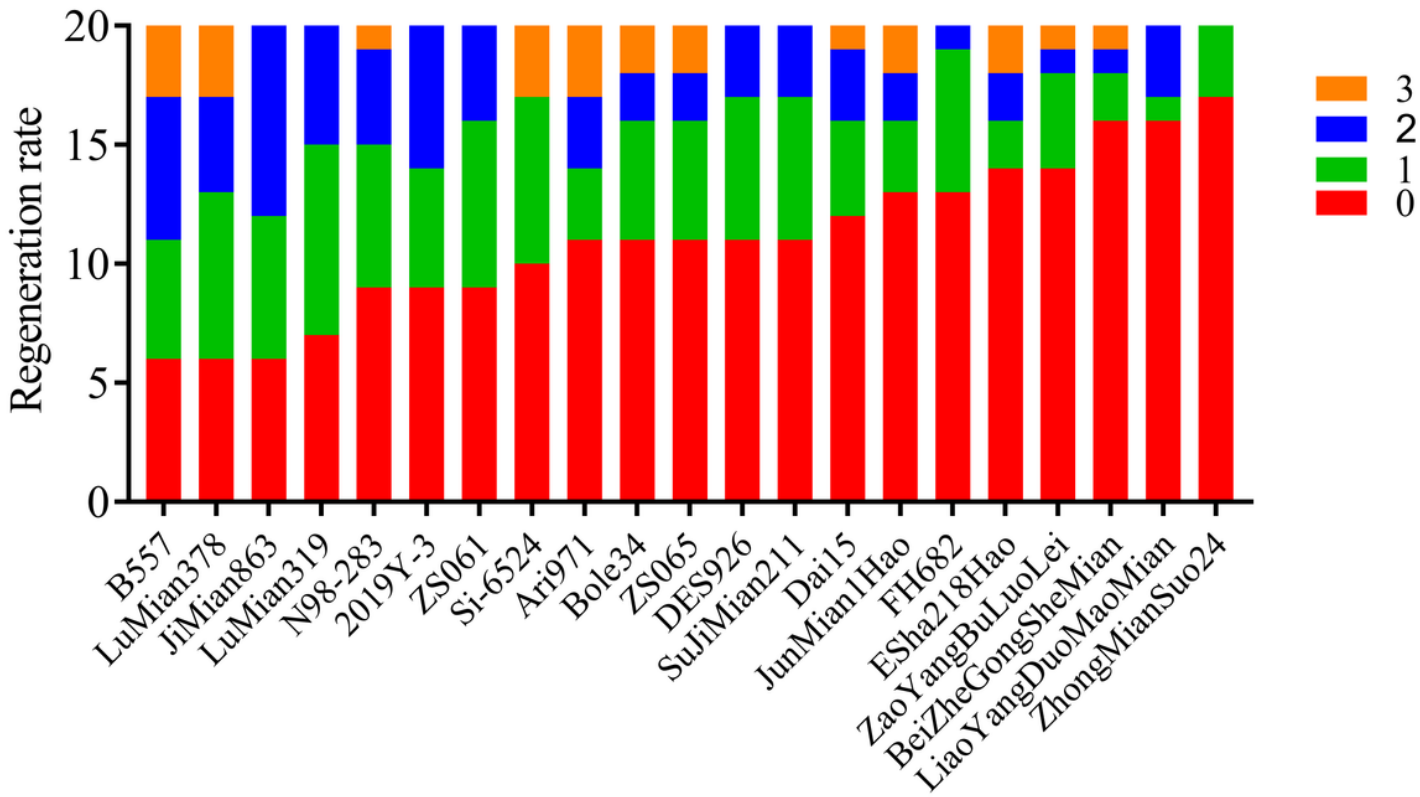

Figure 4

Variation in the field regeneration level among 21 accessions.

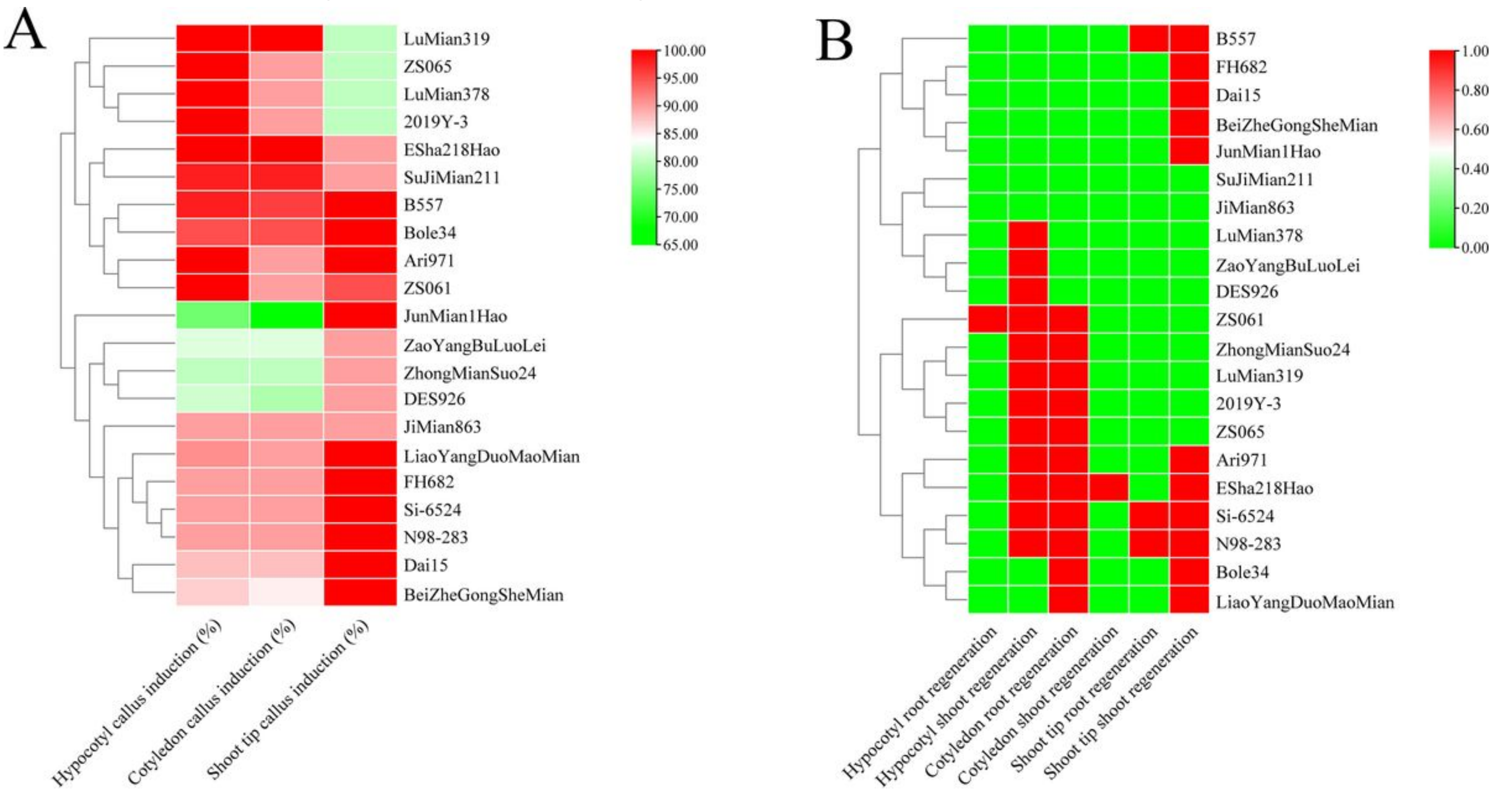

Figure 5

Variation in callus induction percentage and regeneration capacity the number of shoots and roots regenerated on hypocotyl, cotyledon, and shoot tip in 21 accessions.

Supplementary Files

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