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Validating field regeneration capacity for selected accessions of *Gossypium hirsutum* using callus induction and regeneration capacity

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Abstract

Background *Gossypium hirsutum* undergoes rapid clonal propagation to regenerate a mature plant through tissue culture. However, the correlation between cotton leaf regeneration, callus induction, and regeneration ability was still obscure. 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. Agronomic traits, including plant height, leaf area, fresh leaf weight, dry leaf weight, the number of flowers and bolls, and biochemical (proline content) and physiological (chlorophyll and carotenoid content) traits during the flowering stage of 21 upland cotton accessions, were investigated.

Result A significant correlation between physiological parameters and callus induction was discovered. Callus induction and regeneration capacity of roots and shoots for hypocotyl, cotyledons, and shoot tip tissues were used to validate field leaf regeneration level after the first harvest. CCRI 24 showed significant leaf regeneration in the field and callus induction capacity through callus induction and regeneration.

Conclusion We found a substantial relationship between field regeneration capability and callus induction with its regeneration capacity for the hypocotyl, cotyledons, and shoot tip. The results showed that ZS061, Lumian 378, Jimian 863, and ZS065 have the highest moisture retention capacity, while CCRI 24, Liaoyang Duomaomian, and Beizhe Gongshemian have the lowest moisture retention capacity. CCRI 24 has the highest leaf regeneration capacity in the field, while Beizhe Gongshemian has the lowest leaf regeneration capacity. All our result provides a clue for checking the regeneration capacity through leaf regeneration level in the field.

Keywords Upland cotton, Agronomic traits, Biochemical traits, Tissue culture, Callus induction, Regeneration capacity

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Introduction

Cotton (*Gossypium spp.*) is a textile crop worldwide grown. It has 52 species, mostly in the subtropical and tropical areas. Cotton is also an important oilseed crop with high economic value (Chen et al. 2007). Upland cotton (*Gossypium hirsutum*) is grown in roughly 5% of the world's total arable land (Dai et al. 2015; Yu et al. 2016). Cotton is widely regarded as a source of protein, edible oil, and natural fiber (Bello et al. 2022; Tajo et al. 2022). During the growing season, cotton plants shed their leaves in response to drought, disease, nutrient imbalance, frost, or other environmental stresses. However, when the conditions improve, new leaves tend to regenerate. This is a sign of cotton's tolerance and the ability to adapt to new environmental conditions (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 & Huang 2014). Every species has unique regenerative abilities; each organ within a single organism can uniquely respond to regeneration (Oki et al. 2006). Tropical cotton crops can have diverse reactions to insect damage and appear to recover more quickly when subjected to some environmental stresses (Yeates et al. 2010). In addtion, after the first harvest or a drop in temperature, some cotton varieties will 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 varied ratios of auxin and cytokinin. De novo organogenesis, or the synthesis of a new plant from separated organs, can often contribute to establishing a new plant at a wound site without callus formation. *Arabidopsis thaliana* is a model plant used to explore de novo organogenesis (Liu et al. 2014). Studies on proline as a useful solute that enables plants to enhance cellular osmolarity under water stress have been motivated by the long-standing observation that proline accumulates in plants undergoing water stress (Tian et al. 2018). Changes in the protein expression pattern, the attachment of cells to their extracellular matrix substrate, and the pace of cell proliferation and tissue regeneration were frequently caused by carotenoids (Luca et al. 1991). It was reported that proline effects on in vitro callus induction and subsequent regeneration were examined, along with the development of highly reliable and effective plant regeneration technique. It was determined that the medium supplemented with proline was superior to the medium deficient in proline. The best callusing was observed on Murashige and Skoog (MS) medium enriched with 2.0 $mg \cdot L^{-1}$ 2,4-D, 500 $mg \cdot L^{-1}$ proline, and 500 $mg \cdot L^{-1}$ glutamine. The callus formed from media enriched with 500 $mg \cdot L^{-1}$ proline had a higher shoot induction rate (Pawar et al. 2015).

Cold, wet weather, soil crusting, seedling diseases, and hail or wind damage are early-season variables that could hamper cotton germination and emergence. Furthermore, little is known about the ability of damaged seedlings to recover after partial or complete loss of leaf (Longer & 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 as well as de novo formation of various plant structures in *G. hirsutum*, from in vitro explant cultures in response to phytohormones or abiotic stresses, which is referred to regeneration and has a variety of biotechnological applications. To validate the field regeneration potential of *G. hirsutum*, tissue culture studies are required, and efficient rooting is just as important as achieving the highest shoot induction and regeneration response. Using in vitro cultures of different tissues, various cotton cultivars have been investigated for direct or indirect regeneration and callus induction. In upland cotton, the solid–liquid alternating culture method could speed up callus induction and reduce the time to regenerate new tissues (Liu et al. 2020).

Numerous studies have induced various explants into calli, including hypocotyl, cotyledon, root, leaf fragments, and nodal explants in upland cotton (Surgun et al. 2014). The regeneration of shoots from hypocotyl and cotyledon explants was carried out, and the maximum average number of shoots per explant was obtained in hypocotyl explants (Kumar & Srivastava 2015). Excised calli were subcultured on MS media containing appropriate growth hormone (Cheruvathur et al. 2010). These explants produced a smooth and green callus (Mungole et al. 2011). Segments of a leaf placed on a medium for regeneration longer than five days maintain the ability to produce shoots (Subban et al. 2020). Leaf explants were grown on the MS medium containing 4 $mg \cdot L^{-1}$ IBA and 0.5 $mg \cdot L^{-1}$ NAA (Sun et al. 2008). The cotyledon callus induction rate reached 87% (Rahman et al. 2004).

In addition, some studies reported shoot regeneration in a few species (Cheruvathur et al. 2010; Mungole et al. 2011; Subban et al. 2020; 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 result in cell differentiation and the formation of somatic embryos, and leading to the formation of more new cells and tissue regeneration when apical meristem, shoot apex, and cotyledon nodes have been used as explants (Loyola-Varsss et al. 2019; Yavuz et al. 2020). Many studies have revealed the mechanisms of plant regeneration ability at the molecular level in upland cotton. However, evaluating field leaf regeneration capacity about callus induction of upland cotton still needs to be 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

Plant materials

Seeds of 21 varieties of *G. hirsutum* were obtained from the National Mid-term Gene Bank of Cotton, Institute of Cotton Research, Chinese Academy of Agricultural Sciences (ICR-CAAS, Anyang, Henan, China) (Table S1).

Field experiment

Basal synthetic fertilizer (1 000 kg·hm^{-2}; N: P_2O_5: K_2O = 1:1.5:1.5) was applied to the soil before sowing. On May 25, 2020, the seeds were sown at the Experimental Station of ICR-CAAS in Anyang, Henan. The planting row is 7 m long, each spaced 30–40 cm apart. Three replicates were used in the field experiments. Field management followed local recommendations. We investigated the number of germinated plants two weeks after sowing and determined the germination percentage (GP) of each accession as follows:

$$GP = n/N \times 100$$

where, n: the number of germinated plants; N: total number planted in the field,

Phenotype observations

For each of the 21 accessions, the number of flowers and bolls (NFB), leaf area (LA), plant height (PH), fruit branch number (FBN), and leaf branch number (LBN) were recorded. For each trait, we observed 20 plants. The plant height was measured using a 150 cm meter ruler, and the leaf area was measured using a Vernier caliper.

Moisture contents determination

Each mature leaf was removed from 20 plants for each accession during the flowering stage. After we recorded the fresh weight (FW), leaves were aired to dry for two days and recorded the dry weight (DW), and the leaf weight lost (LWL) was calculated as LWL = FW-DW.

Relative electronic conductivity (REC) analysis

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

Determination of total carotenoid content

Leaves from 20 plants for each accession were cut into smaller sizes (0.5 g) and ground with a mortar and pestle with 10 mL of acetone (80%); the extract was centrifuged at 2 500 r·min^{-1} for 10 min and made up to 10 mL with 80% distilled acetone. The absorbance of the extract was read at 480 nm, 510 nm, and 750 nm, respectively. The total amount of carotenoids (CA, mg·g^{-1}) was calculated by using the following formula (Tian et al. 2023):

$$CA = \frac{(7.6 \times (OD_{480} - OD_{750}) - 1.49 \times (OD_{510} - OD_{750})) \times V}{FW \times 1000}$$

where V: final volume of supernatant (mL); FW: fresh weight of the leaf sample (g).

All the analyses were carried out in three replicates for each plant.

Determination of chlorophyll content and total proline content

SPAD-502 was used to measure the chlorophyll content in matured leaves in three portions for three different leaves of each plant (León et al. 2007). Twenty plants of each accession have been measured. Proline content was also extracted and estimated using a cold extraction procedure, once with 80% ethanol and once with 50% ethanol. The cold extraction method can be repeated on the pellet, and the supernatants can be combined and used for analysis (Bates et al. 1973).

Field regeneration level investigation

The first harvest was done 150 days after sowing. After the harvest, newly growing leaves were counted and recorded one by one for 20 plants of each accession. The field regeneration capacity was represented as follow:

$$\text{Regeneration rate} = N_0 + N_1 + N_2 + N_3$$

N_0 means the number of plants that did not produce a new leaf; N_1 means the number of plants that produced one new leaf; N_2 means the number of plants that produced two new leaves; N_3 means the number of plants that produced three or more new leaves.

Callus induction and tissues regeneration capacity

Cotton seeds from all accessions were delinted and sterilized with 0.1% $HgCl_2$ solution. The seed was shaken for eight minutes before being rinsed with sterilized distilled water five times for one minute each time. The sterilized seeds were placed on a plate and put into a dark growth chamber at 28 °C for 48 h. The young germinating seedling was transplanted into bottles and placed in a dark chamber set at 28 °C for five days. The seven-days-old seedlings were cut into hypocotyl, cotyledon, and shoot tips and plated in MS medium for callus induction. Each explant size range is approximately 1 cm. Based on the possibility of generating different calli for different tissues, hypocotyl, and cotyledon were transplanted into MS medium containing 2,4-D (0.5 $mg \cdot L^{-1}$) and kinetin (0.1 $mg \cdot L^{-1}$), respectively. Shoot tips were transplanted into MS medium containing 2,4-D (0.5 $mg \cdot L^{-1}$) and kinetin (0.2 $mg \cdot L^{-1}$) (Chakraborty & Banerjee, 2013). All tissues were placed in a light chamber (16 h of light and 8 h of darkness) at 28 °C for 14 days, and the callus induction rate was calculated as follows:

$$CIR_{2w} = \delta y / \delta x \times 100$$

where CIR_{2w}: two weeks' callus induction rate; δy: total number of callus tissue formed within two weeks per plate; δx: total number of tissues in plate within two weeks.

Two weeks later, all the tissues were switched to a new MS medium with the same phytohormone supplement and growth for another two weeks, during which the callus induction rate was monitored and documented once more, and the callus induction rate was calculated as follows:

$$CIR_{4w} = \delta y / \delta x \times 100$$

where CIR_{4w}: four weeks' callus induction rate; δy: total number of callus tissue formed within two weeks per plate; δx: total number of tissues in plate within two weeks.

Plates with good callus induction were selected to observe whether shoots and roots from three different tissues might regenerate.

Results

Agronomic trait analysis

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

Plant height

The plant height of all 21 accessions has a significant variation (from 51.65 cm to 99.85 cm) according to an analysis of variance ($P < 0.01$). Accession with the highest plant height is Lumian 378, and Junmian 1 with the lowest plant height (Fig. 1).

Fruit branch number

Fruit branch number is an important trait for fiber yield in cotton. Five accessions, including Ari 971, Si-6524, B557, DPL 15, and Bole 34, have more fruit branch numbers (from 13.05 to 13.74) than the other three accessions ZS065, Sujimian 211, and ZS061 (9.7, 9.3, and 9.05, respectively. Fig. 1).

The number of flowers and bolls

The comparison between flower and boll number results revealed that five accessions Bole 34, Ari 971, DPL 15, Esha 218, and DES926 have the highest number of flowers and bolls (from 15.35 to 18.10). However, FH682 accordingly exhibits the lowest number of flowers and bolls (3.95) (Fig. 1).

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 $P < 0.01$. Ari 971 and Si-6524 have the highest LBN (13.55 and 12.45, respectively). Six accessions with the lowest LBN were Junmian 1, Beizhe Gongshemian, Liaoyang Duomaomian, DPL 15, ZS061, and Esha 218 (from 4.55 to 9.3) (Fig. 1).

Leaf area and leaf weight lost

Leaf area (LA) directly impacts the photosynthetic rate of cotton plants. According to the variance analysis result, ZS061, Lumian 378, Jimian 863, and ZS065 have higher LA and LWL values. In contrast, Junmian 1, CCRI 24, Liaoyang Duomaomian, and Beizhe Gongshemian have lower LA and LWL values. These results indicated that

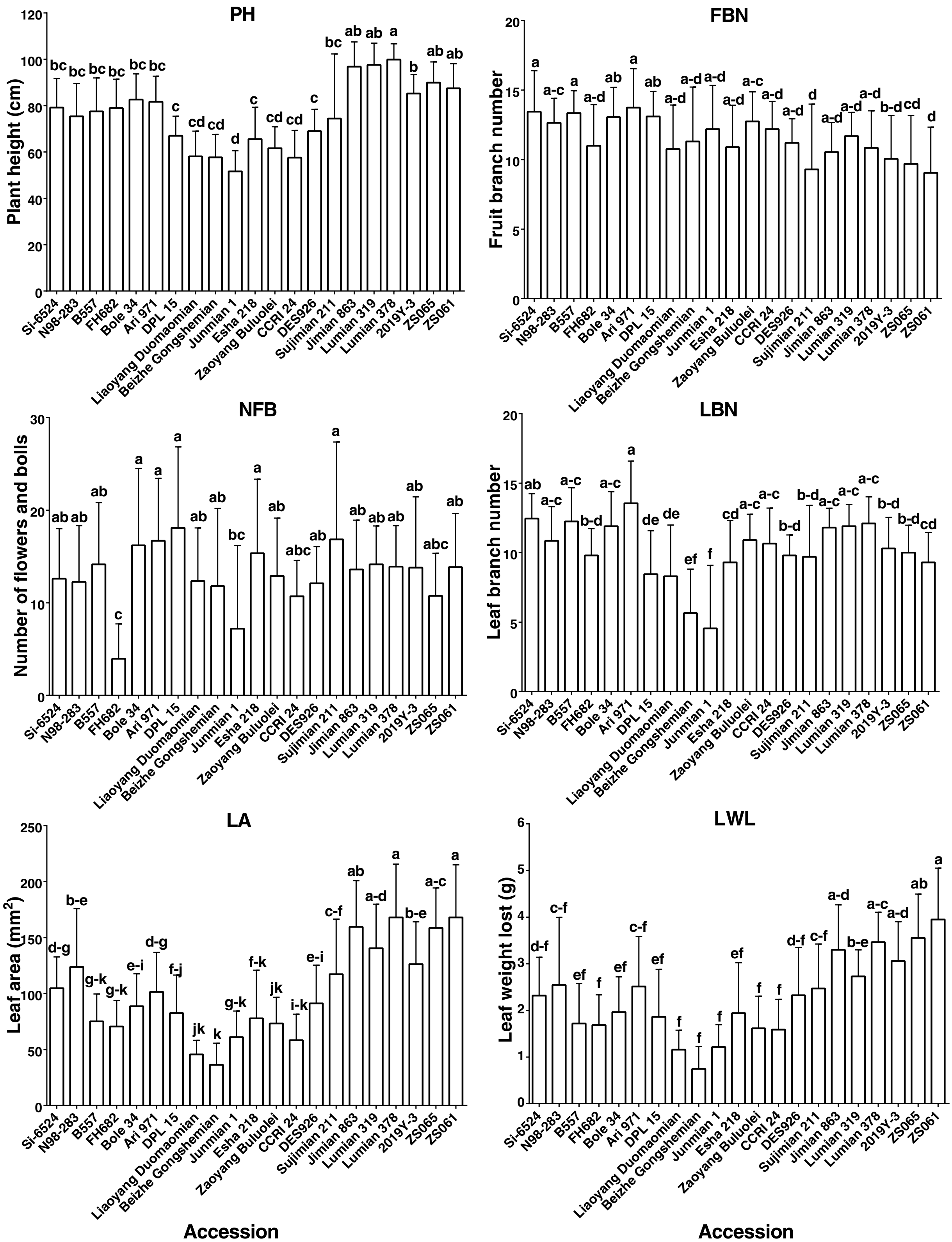

Fig. 1 Variation analysis on six agronomic traits in 21 accessions. Using one-way ANOVA, bars with different letters indicate a significant difference ($P < 0.05$). PH (Plant height), FBN (Fruit branch number), NFB (Number of flowers and bolls), LBN (Leaf branch number), LA (Leaf area), LWL (leaf weight lost)

cotton plants with large leaves' surface area easily lost moisture and dried off.

Biochemical analysis

High temperature and drought stress could degrade cell membrane structure, cause proline synthesis, reduce chlorophyll concentration, and result in leaf senescence. Ion leakage is crucial in determining cell membrane stability and can be used to assess abiotic stress tolerance such as water and heat stress.

The result showed that three accessions, Bole 34, N98-283, and FH682 (from 4.95% to 5.65%), were more tolerant than others, especially Jimian 863 (22.24%) and Lumian 319 (21.32%) (Fig. 2).

Chlorophyll and carotenoid content were correlated to the photosynthetic potential of plants and gave some indication of the physiological status (Gamon et al. 1997). The chlorophyll contents of Sujimian 211 and 2019Y-3 were significantly higher than Zaoyang Buluolei, B557, and Jummian 1. However, the changing trend of the carotenoid content in all 21 accessions was different. N98-283, ZS061, Si-6524, and CCRI 24 were significantly higher than other accessions (Fig. 2). Biochemical parameters, such as antioxidant

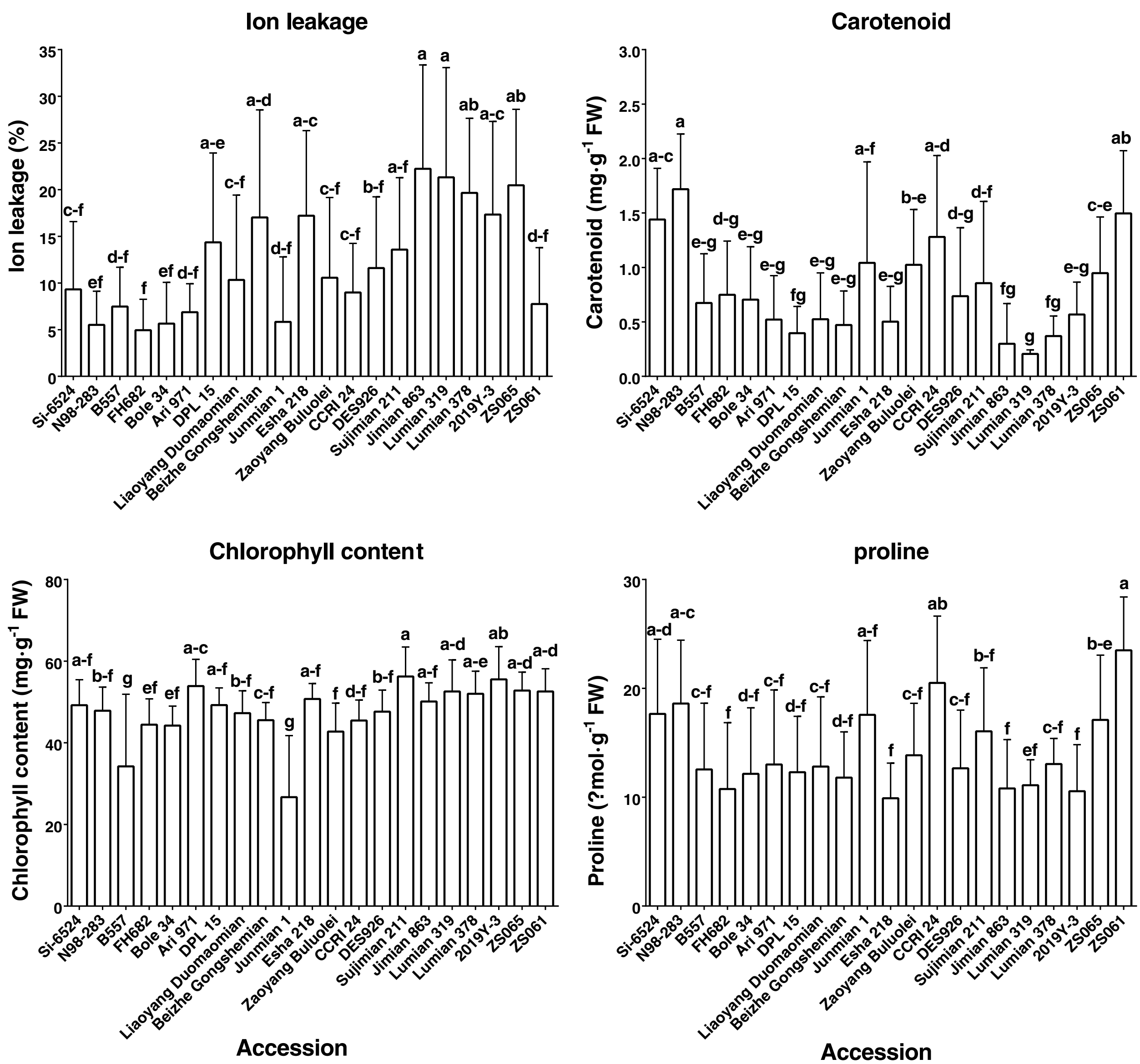

Fig. 2 Variation analysis on biochemical traits for 21 accessions. Bars with different letters indicate a significant difference ($P < 0.05$) using one-way ANOVA

enzyme activities and proline content, were chosen to examine cotton plants' reactions to drought stress (Chen et al. 2016).

Proline content is another important parameter for abiotic stress tolerance. ZS061, CCRI 24, N98-283, and Si-6524 showed higher proline contents than others (Fig. 2).

Regeneration capacity analysis by field investigation

Some lines of upland cotton produce new leaves after harvest when the temperature drops, and old leaves tend to fall away. These emergences of new leaves have been categorized into four levels (Fig. 3). The first category (level 3) produced plenty of new leaves, which has three new leaves and above; the medium level (2), which has two new leaves; level 1 which has 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. The result showed that CCRI 24, B557, and Lumian 378 have higher regeneration rates, and Liaoyang Duomaomian, Beizhe Gongshemian, and Zaoyang Buluolei have lower regeneration rates. Other accessions have medium regeneration rates (Fig. 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 rates and regeneration capacities (shoot and root regeneration) were analyzed and recorded (Fig. 5).

The first clade, which includes Lumian 319, ZS065, Lumian 378, and 2019Y-3, has higher hypocotyl and cotyledon callus induction rates. The second clade, which includes six accessions, showed higher callus induction rates in three tissues, but the levels were different. Esha 218 and Sujimian 211 have lower callus induction rates in shoot tips than in the hypocotyl and cotyledon, but Ari 971 and ZS061 have lower callus induction rates in the cotyledon than in the hypocotyl and shoot tips. B557 and Bole 34 have significantly higher callus induction rates in both tissues. The third clade, including four accessions (Junmian 1, Zaoyang Buluolei, CCRI 24, and

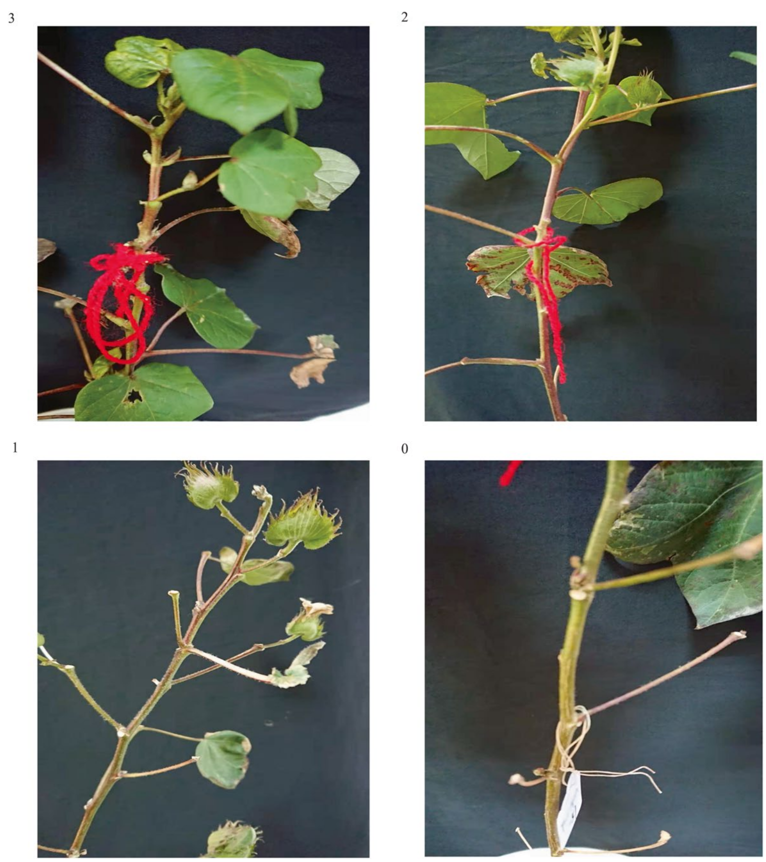

Fig. 3 Different field regeneration levels. N_0: the number of plants that did not produce a new leaf; N_1: the number of plants that produced one new leaf; N_2: the number of plants that produced two new leaves; and N_3: the number of plants that produced three or more new leaves

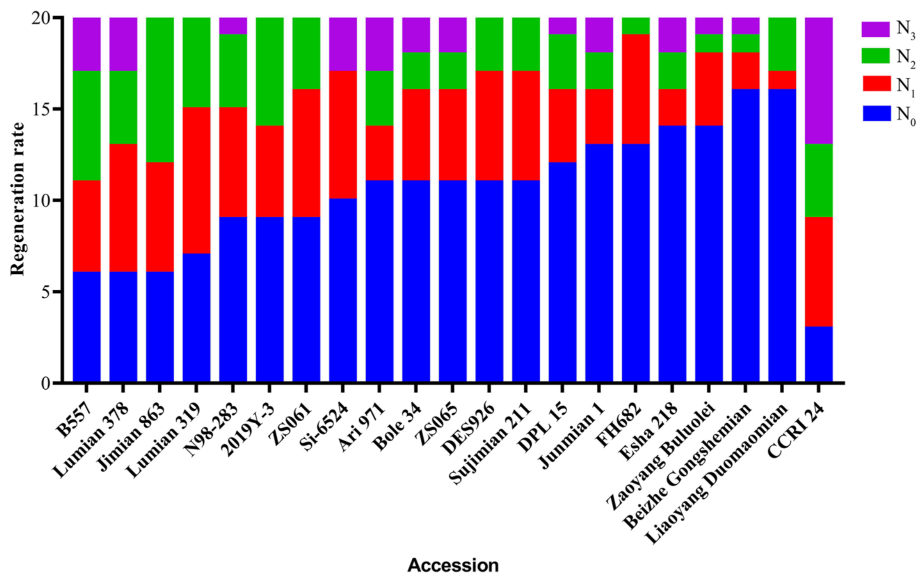

Fig. 4 Variation in the field regeneration level among 21 accessions

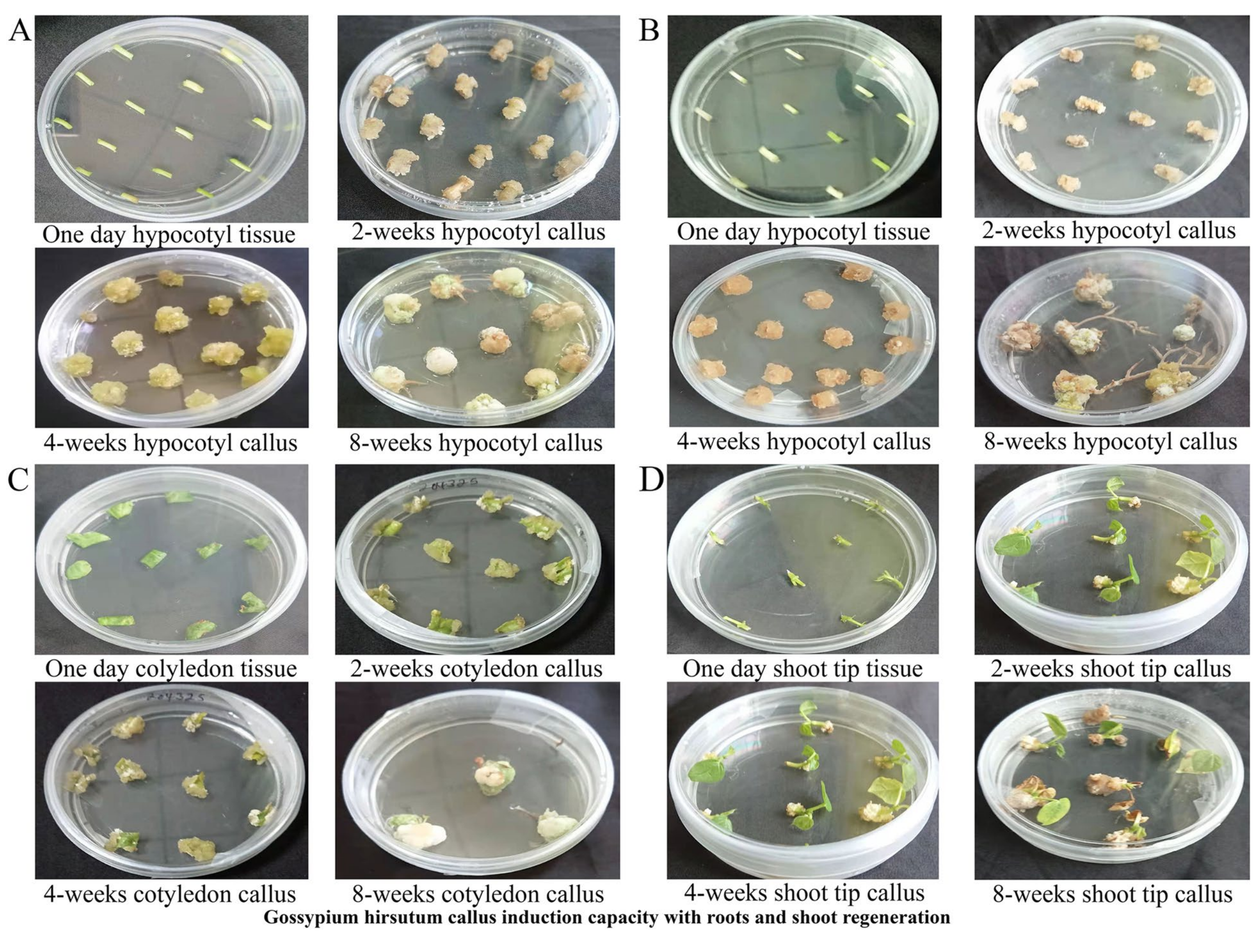

Fig. 5 Shoot and root regeneration during the callus induction period in different tissues. (**A**) One day hypocotyl, two weeks hypocotyl callus, four weeks hypocotyl callus, and eight weeks hypocotyl callus in Lumian 319. (**B**) One day hypocotyl, two weeks hypocotyl callus, four weeks hypocotyl callus, and eight weeks hypocotyl callus in Esha 218. (**C**) One day cotyledon, two weeks cotyledon callus, four weeks cotyledon callus, and eight weeks cotyledon callus. (**D**) One day shoot tip callus, two weeks shoot tip callus, four weeks shoot tip callus, and eight weeks shoot tip callus

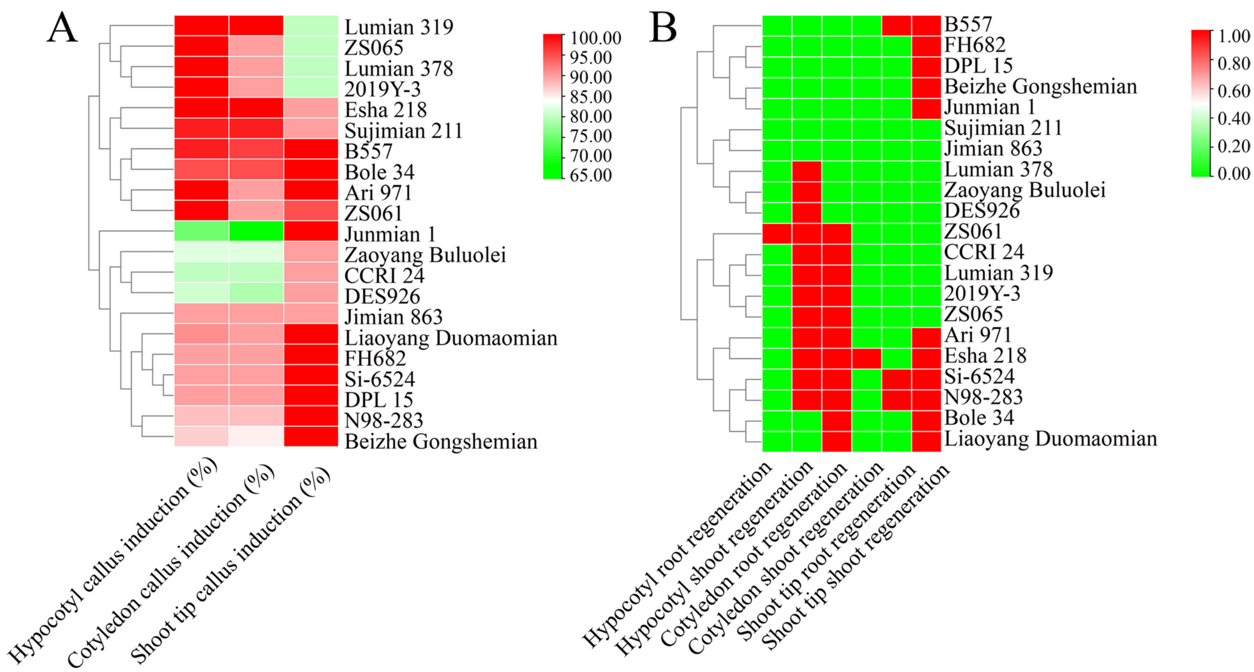

Fig. 6 The number of shoots and roots regenerated on hypocotyl, cotyledon, and shoot tip in 21 accessions varies in callus induction percentage and regeneration capacity variation

DES926), showed significantly lower callus induction rates in the hypocotyls and cotyledon than in the shoot tip. The fourth clade includes Jimian 863, which has a medium callus induction rate in both tissues. The last clade includes five accessions that have higher callus induction rates in the shoot tip than in the hypocotyl and cotyledon. From these results, it is known that one accession has different callus induction rates in different tissues, and those accessions in clades 2 and 5 could be used to do the next callus induction experiment and so on (Fig. 6A).

During the callus induction period, some tissues have shoot and root growth (Fig. 6B). For hypocotyl tissue, only one accession ZS061 regenerated a new root, while 12 accessions (Lumian 378, Zaoyang Buluolei, DES926, ZS061, CCRI 24, Lumian 319, 2019Y-3, ZS065, Ari 971, Esha 218, Si-6524, and N98-283) were observed to have new shoot regeneration in the hypocotyl. In cotyledons, 11 accessions (ZS061, CCRI 24, Lumian 319, 2019Y-3, ZS065, Ari 971, Esha 218, Si-6524, N98-283, Bole 34, and Liaoyang Duomaomian) were observed to have root generation, but only Esha in 2018 has shoot regeneration. Three accessions (B557, Si-6524, and N98-283) have root regeneration, and 11 accessions (B557, FH682, DPL 15, Beizhe Gongshemian, Junmian 1, Ari 971, Esha 218, Si-6524, N98-283, Bole 34, and Liaoyang Duomaomian) have shoot regeneration in the shoot tip tissue. Two accessions, Sujimian 211 and Jimian 863, did not grow shoot or root in all three tissues. From this result, hypocotyls and shoot tip tissues tend to generate shoot during the callus induction period, while the cotyledon generates root, and the regeneration capacity for different accessions varied.

A significant correlation was found between callus induction and new tissue regeneration (shoots and roots). There is also a significant correlation between the shoot tip callus induction, shoot tip roots, and shoot regeneration. This correlation was also observed in hypocotyl tissue. For example, there is a significant correlation between shoot tip callus induction and shoot regeneration capacity on Junmian 1 (Table 1).

Discussion

In this study, we carried out phenotypic, physiological, and biochemical analyses of 21 upland cotton accessions. Also, we used the callus induction rate and the regeneration of shoots and roots to validate the field regeneration capacity of each accession. The plant height of cotton results from 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 the results, three accessions (Jimian 863, Lumian 378, and Lumian 319) were discovered to have higher plant height than other accessions. Light is a source of energy and an important signal for environmental changes,

Table 1 Correlation analysis of callus induction data and regeneration levels

Correlation Trait	Hypocotyl CI	Cotyledon CI	Shoot tip CI	Hypocotyl RR	Hypocotyl SR	Cotyledon RR	Cotyledon SR	Shoot tip RR	Shoot tip SR	Little NLP	Medium NLP	Plenty NLP
Hypocotyl CI	1											
Cotyledon CI	0.91**	1										
Shoot tip CI	-0.13	0.01	1									
Hypocotyl RR	0.16	0.20	-0.08	1								
Hypocotyl SR	0.60**	0.48**	-0.28	0.18	1							
Cotyledon RR	0.35*	0.28	-0.12	0.24	0.51**	1						
Cotyledon SR	0.33*	0.43**	0.02	0.42*	0.12	0.09	1					
Shoot tip RR	0.18	0.30	0.49**	-0.07	-0.05	-0.03	0.19	1				
Shoot tip SR	0.20	0.29	0.54**	0.18	0.22	-0.01	0.15	0.15	1			
Little NLP	0.12	0.08	-0.15	0.08	0.02	-0.10	-0.09	-0.29	0.09	1		
Medium NLP	0.18	0.21	0.23	0.20	0.24	0.12	0.09	0.05	0.37*	0.01	1	
Plenty NLP	-0.16	-0.21	0.21	-0.07	0.13	0.09	-0.08	0.15	0.09	-0.09	0.26	1

CI Callus induction, *RR* Root regeneration, *SR* Shoot regeneration, *NLP* New leaves produce

*, ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively

causing various physiological reactions in plants (Abidi et al. 2013). Previous reports revealed that photoperiods significantly influenced photosynthesis, seed germination, breaking of dormancy, and flowering (Rezazadeh & Harkess 2018; Skjelva 2004). Our findings revealed that there is a significant correlation between plant height and leaf area on ZS065 accession; plant height has a significant role in the growth and development of upland cotton due to the large leaf area trapping 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 positively correlated with maximum plant height. In herbaceous grassland species, taller species often flower later than shorter ones. In the Jimian 863 accession, we discovered a significant relationship between leaf area and plant height and its callus induction has new shoot regeneration. Less leaf area and leaf branches were observed in Junmian 1 accession, corresponding to a high carotenoid content level. This could be due to the less amount of sunlight striking 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).

Because this study had only one-year field results about regeneration, we used callus induction and shoot and root regeneration to validate the field results. Table 1 shows a significant correlation between callus induction and new tissue regeneration (shoots and roots). There is also a significant correlation between shoot tip callus induction, shoot tip roots, and shoot regeneration. This correlation was also observed in hypocotyl tissue. For example, there is a significant correlation between shoot tip callus induction and shoot regeneration capacity on Junmian 1. Low moisture content and low conductivity were observed in the Junmian 1 accession. The high percentage of re-generable calli is a prerequisite for genetic manipulation toward varietal improvement. This study aimed to find accessions with efficient callus induction and regeneration for better plant regeneration efficiency in the field, according to Fig. 3. The number of flowers and bolls is an important trait for upland cotton. More bolls produce enormous fiber content. Two accessions (Bole 34 and DPL 15) were found to have higher number of flowers and bolls, and produce new shoots and roots in cotyledon, and shoot tip tissues. This indicates that good callus induction induces new tissue regeneration in the shoot tip and cotyledon tissues of Bole 34 and DPL 15 accessions.

Moderate callus induction was observed in the shoot tip tissue of Lumian 319, which correlates significantly with shoot regeneration. Bole 34 has the highest shoot-tip callus induction (100%), which correlates with root regeneration of cotyledon and shoot generation from the hypocotyl and shoot tip. Multiple shoot and root regeneration correlate with the maximum callus induction capacity. In the field, it was also discovered that two accessions, Junmian 1 and FH682, had limited or less flower and boll, and some accessions did not produce a new leaf. Callus induction capacity confirmed these accessions' new tissue regeneration levels. Shoot tip callus induction with corresponding shoot regeneration was observed in Junmian 1. Less leaf branches, less moisture content, and less conductivity were observed in Junmian 1; this may be attributed to the effects of moisture on conductivity level. Shoot tip callus induction (100%) correlates with good shoot regeneration capacity in FH682. In hypocotyl callus induction, a high callus induction rate (100%) was observed in 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 the hypocotyl in seven accessions (ZS061, Ari 971, Esha 218, 2019Y-3, Lumian 378, ZS065, and Lumian 319). It was also discovered that Junmian 1 with less flower and boll numbers in the field correlates with a low callus induction level and low regeneration capacity in hypocotyl tissue. The same accession also displayed a limited response to regeneration capacity. Our findings also revealed that the genotype greatly influences callus induction abilities and agrees with the previously reported work in *Oryza sativa* (Abe & Futsuhara 1986). The composition of the culture medium, genotype, and condition influences callus induction and regeneration rates. The genotype is the most critical factor impacting in vitro culture efficiency. Callus formation and plant regeneration ability in explants of the same age and with the same growth regulator combination is mostly determined by genotype (Arzani & Mirodjagh 1999). The fruit branch number is an important trait for upland cotton; the quality of cotton fiber results from an interaction of environmental, genetic, and management factors, determined by the position of the resources obtained by each fruit (Girma et al. 2007; Percy et al. 2006). Effective leaf regeneration was observed in CCRI 24, possibly due to the ability to withstand drought after harvesting. CCRI 24 has high proline content, and proline serves as a superior osmolyte and functions as a metal chelator, an antioxidant defense molecule, and a signaling molecule under stress (Hayat et al. 2012).

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 & Kuhlemeier 2002; Sakamoto & 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, could indicate the drought resistance levels of each accession. According to the mean comparison values, two accessions (ZS065 and ZS061) have the strongest moisture retention affinity. Similarly, the lowest affinity to moisture retention was observed in Beizhe Gongshemian and Liaoyang Duomaomian. In the Lumian 319 accession, a significant correlation was observed between moisture content and net conductivity. This is likely due to genetic variation in the genome constituent within the *G. hirsutum*. It was reported that plants evolved to regulate growth periods to avoid moisture stress, termed drought escape (Manavalan et al. 2009). The first response of plants to moisture stress as a drought-resistance strategy relies on avoiding water deficit by maintaining tissue weight through increasing water uptake or restricting water loss (Zonta et al. 2017). In our result, leaf conductivity was correlated with higher chlorophyll content, and this is similar to a related finding that photosynthetic stimulation was correlated with increased leaf conductance (Chen, et al. 2018).

Carotenoid content, which functions in trapping the sunlight for photosynthesis, and the values of each accession were measured and recorded. Table S2 showed a significant correlation between carotenoid value and other traits. In rare findings, high carotenoid content correlated with high chlorophyll values in N98-283, ZS061, and Si-6524 accessions. Cotton which attains a required height does not necessarily need high carotenoid content to trap more sunlight to attain maximum growth. Higher carotenoid content was recorded in N98-283, which has a lower plant height compared with other accessions with a lower carotenoid value. Higher carotenoid content does not necessarily correlate with plant height, and this can be supported given the intensity of plant height, which increased at lower light intensity (Wang & Li 2008).

Proline is an important trait in *G. hirsutum* to overcome biotic and abiotic stresses. In comparing proline levels among 21 accessions, we found that Junmian 1 has the highest proline content and low-moderate moisture content. This result supports the finding that plants were subjected to a drying cycle when the plants had four true leaves, and the proline content of the leaf tissue was determined (Chen 1966). There are many significant correlations among the 21 accessions in the callus induction and new tissue regeneration rates. Nine accessions have 100% callus induction capacity on the hypocotyl tissue compared with the regeneration capacity and callus induction on hypocotyl tissue, the callus induction rate was above 50%, as observed in all three tissues (hypocotyl, cotyledons, and shoot tip). The highest callus induction was observed in the shoot tip, possibly due to meristematic cells at the tip end of the seedling in *G. hirsutum*. It was reported that a high percentage of calli is a prerequisite for genetic manipulation toward varietal improvement (Mushke et al. 2016). Callus induction is the best requirement for successful tissue regeneration. Successful callus induction and subsequent tissue regeneration depend on the exogenous supply of plant hormones (Rueb et al. 1994; Samota et al. 2017). It was also reported that the growth and development of the leaves were impacted due to the damage caused by the cold and injury to cotton. After the favorable conditions returned, the leaves actively regrew. This study supports the finding that cotton leaves regenerate (Gao et al. 2021).

Conclusion

In this study, a substantial relationship was found between field regeneration capability and callus induction with its regeneration capacity for the hypocotyl, cotyledons, and shoot tip. ZS061, Lumian 378, Jimian 863, and ZS065 had higher moisture retention capacity, while CCRI 24, Liaoyang Duomaomian, and Beizhe Gongshemian had lower moisture retention. CCRI 24 had the highest leaf regeneration in the field, while Beizhe Gongshemian had the lowest leaf regeneration. All the result provides a clue for checking the regeneration capacity through leaf regeneration level in the field.

Abbreviations

GP	Germination percentage
N	Total number planted in the field
n	The number of germinated plants
NFB	The number of flowers and bolls
PH	Plants height
LA	Leaf area
FBN	Fruit branche number
LBN	Leave branche number
FW	Fresh weight
DW	Dry weight
LWL	Leaf weight lost
CA	Carotenoid
V	Final volume
W	Weight
CIR_{2w}	Two weeks callus induction rate
CIR_{4w}	Four Weeks callus induction rate

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-023-00146-x.

Additional file 1: Supplementary Tables 1. The passport information of germlasm used in this study.

Additional file 2: Supplementary Table 2. Correlation analysis of ten phenotypic and physiological traits.

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Authors' contributions
Du XM and Geng XL conceived and designed the experiments, Tajo SM performed the experiments, Tajo SM and Geng XL analyzed the data and wrote the manuscript, and Mahmood T, Jia YH, and He SP helped with data analysis. Pan ZE, Hu DW, Wang LR, Sadau BS, Ibrahim S, and Auat U helped revise the manuscript. All authors have checked and approved the final manuscript.

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Declarations

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Competing interests
All authors declared that they have no competing interests.

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