The plant regeneration and genetic transformation of *Sapium sebiferum*: an important bioenergy plant

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Outlines

- Backgrounds
- Research progress
- Conclusions

Backgrounds

***Sapium sebiferum* Roxb.**

- **known as tallow tree, popcorn tree**
- **has strong adaptability**
- **a major promising oil-yielding woody species in China**

high oil content in seeds (55%)

biodiesel production

colorful foliage in autumn

Shortages：

- Long juvenile phase
- Low yield
- Highly heterozygous, polyploidy

乌桕核型 Karyotype of *S. sebiferum*

tetraploid

Research progress

- **Plant regeneration of *S. sebiferum* through different pathways**
    1. somatic embryogenesis
    2. direct organogenesis
    3. indirect organogenesis
- **Droplet-vitrification cryopreservation of *S. sebiferum***
- **Genetic transformation of *S. sebiferum***

1. Plant regeneration systems

1.1 Regeneration via Somatic Embryogenesis

Explant : immature zygotic embryo (IZE)

Mechanical damage had great influence on somatic embryogenesis

Fig.1. Embryo germination from intact IZEs

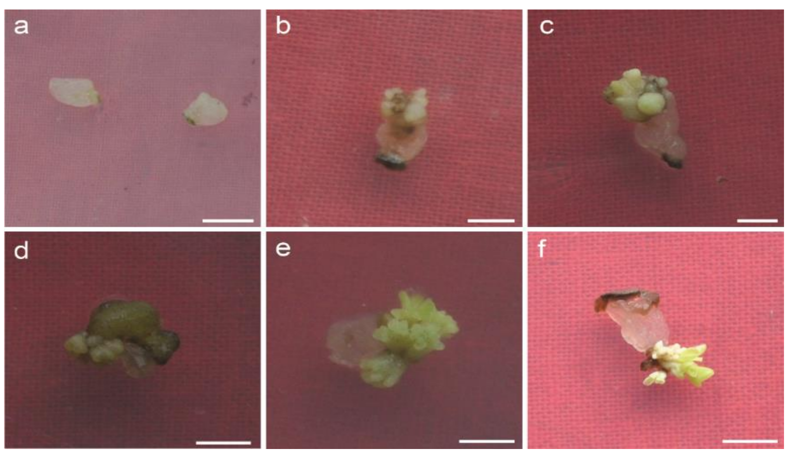


Fig.2. Somatic embryogenesis from wounded IZEs

Auxins also had great influence on somatic embryogenesis

Fig.3

Effect of auxins on the germination of intact IZEs.

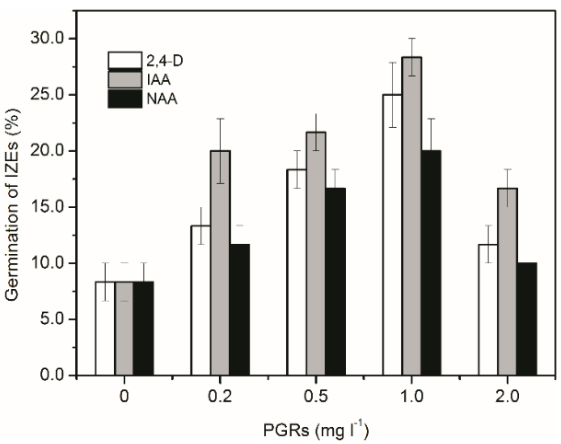


Fig.4

Effect auxins on the induction frequency of SE from mechanical damaged IZEs

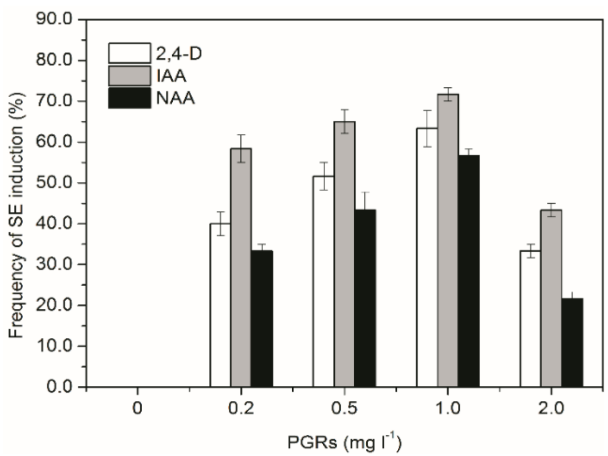


Fig.5

Effect of auxins on the number of SEs produced per explant

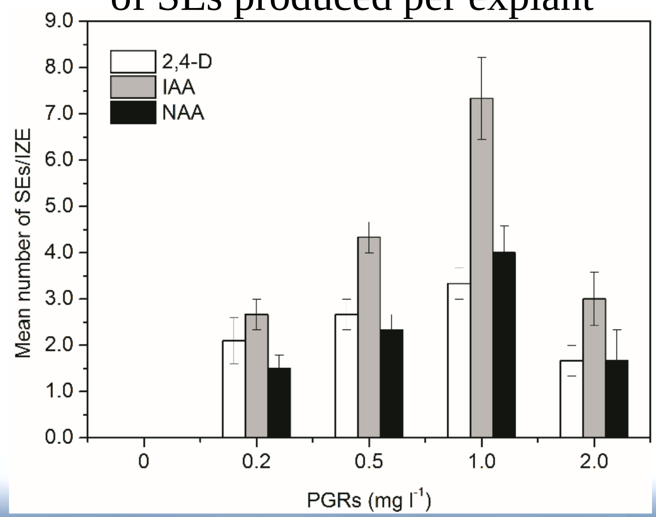
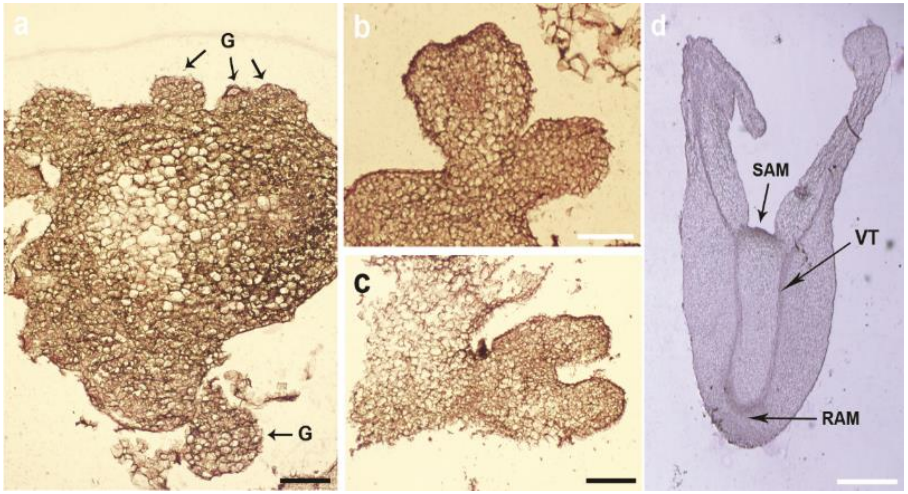
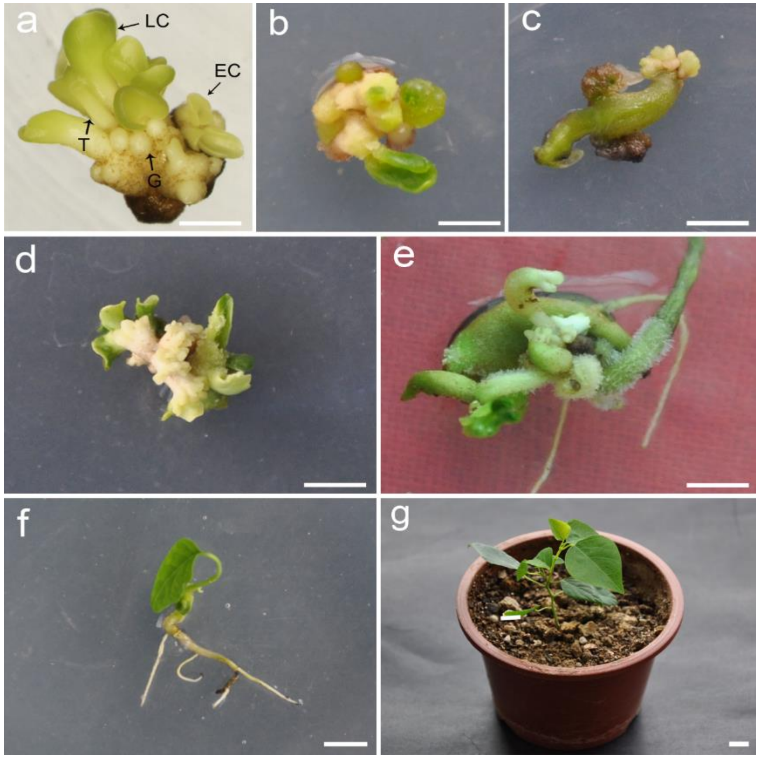


Fig.6 Histological analysis of somatic embryogenesis from IZE.



Globular stage ⟹ heart-shaped stage ⟹ torpedo stage ⟹ cotyledonay stage

Fig.7 Secondary somatic embryogenesis and plant regeneration of S. sebiferum.

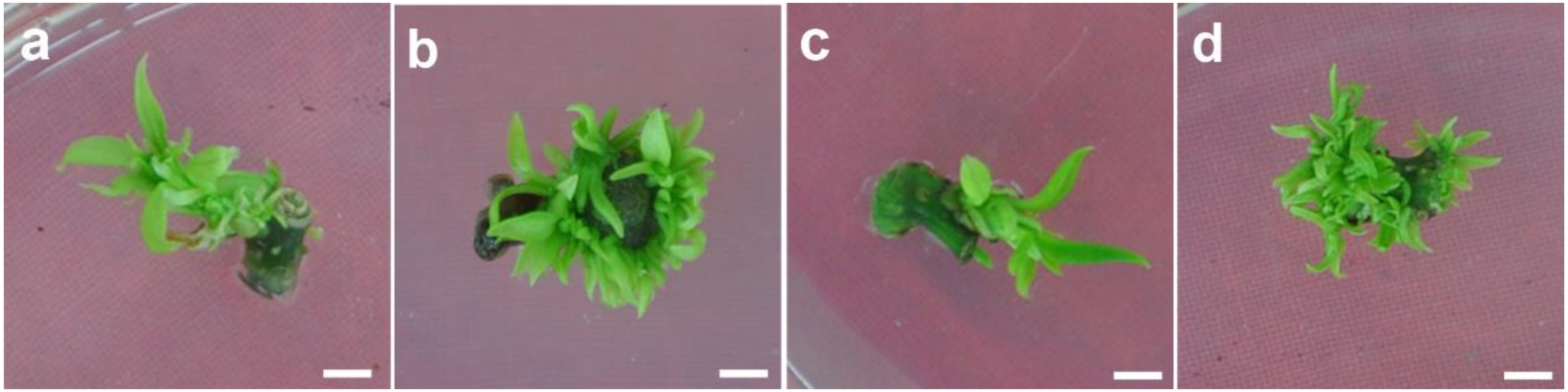


The induction frequency of somatic embryogenesis was up to 90.0% with 13.5 SEs/explant

1.2 Regeneration via direct shoot organogenesis

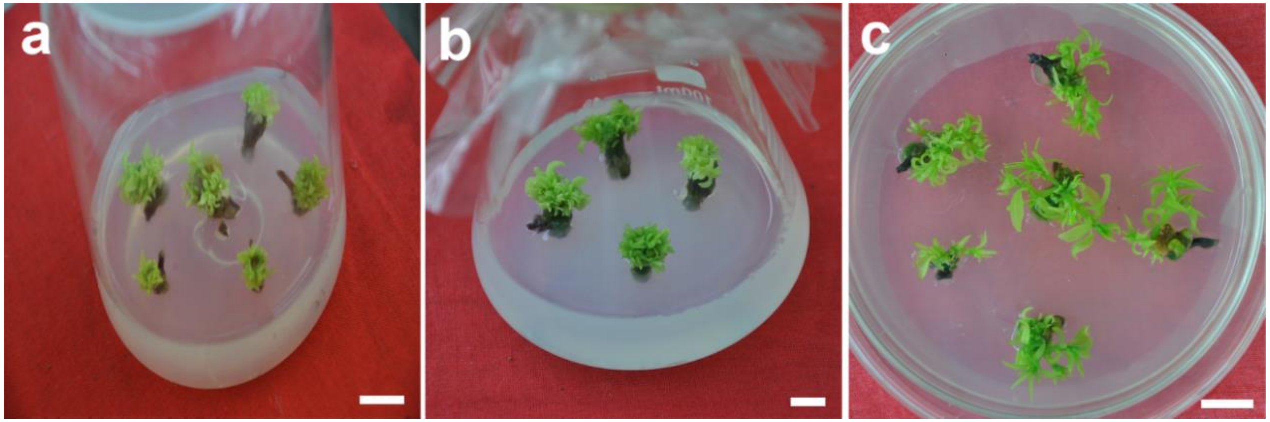
Explant: Shoot stem with axillary bud

Effect of explant orientation and PGRs on adventitious shoot induction

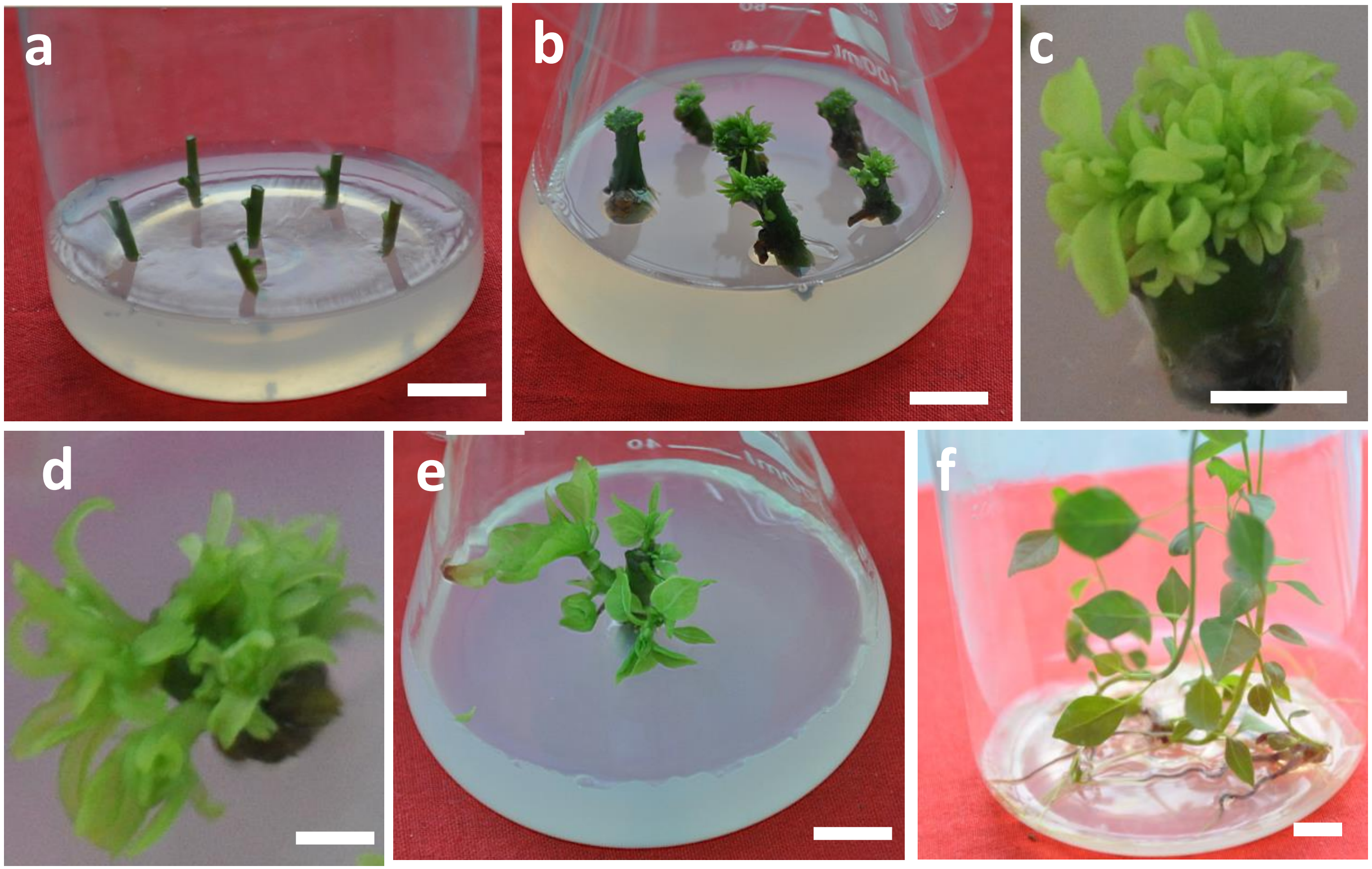


Vertical: a , b; Horizontal: c, d

Effect of culture vessel type on shoot induction

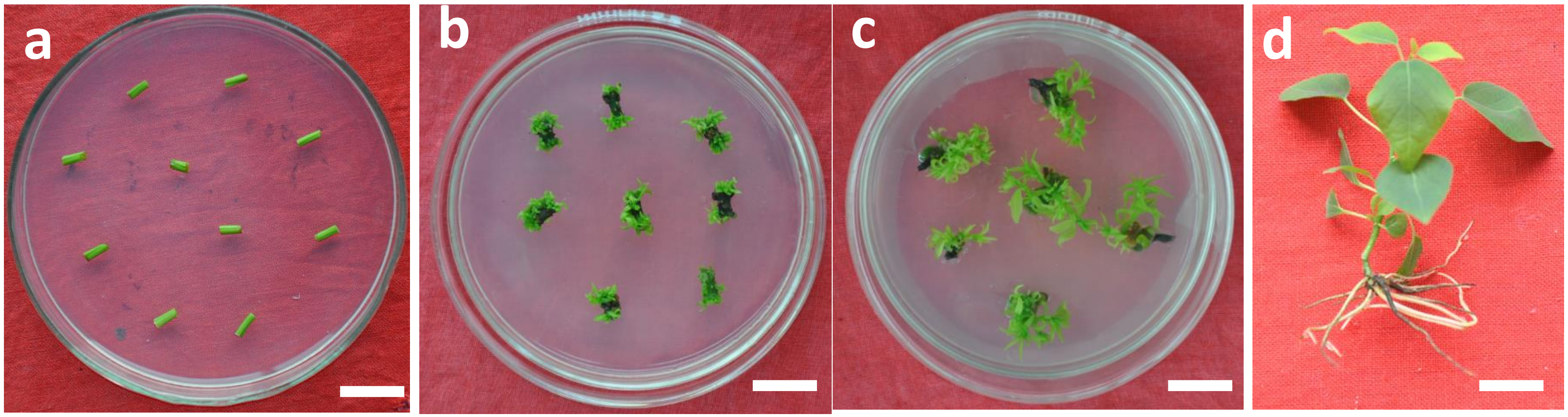


Adventitious shoots regenerated in: a) plastic bottle; b) glass bottle; c) glass petri dish

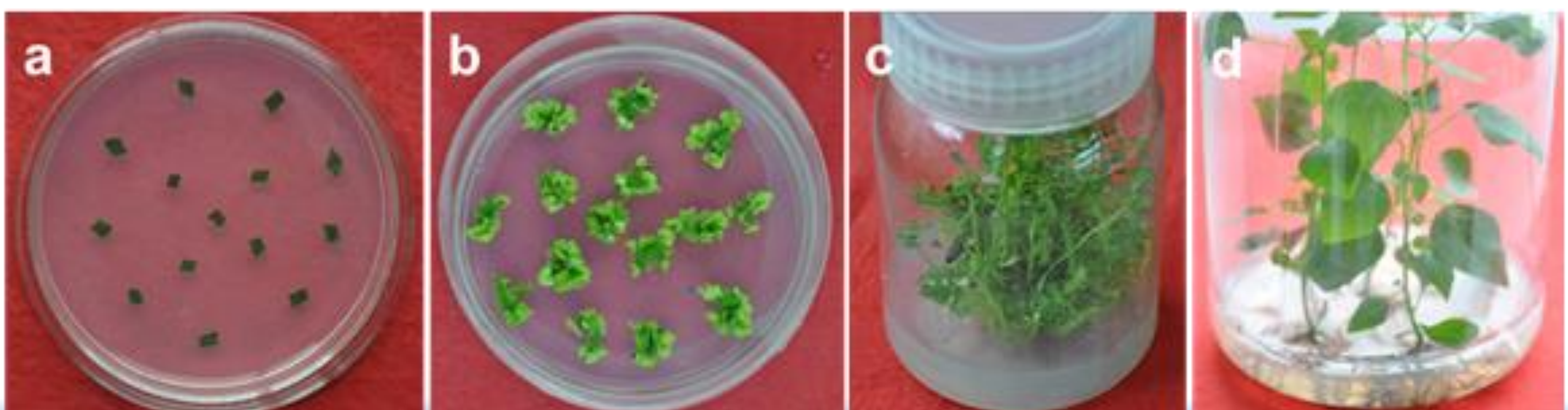


When the nodal stem with axillay shoot placed horizontally on the medium with PGRs in glassic petri dishes, the highest shoot induction frequency reached 93.3 % with 11.1 shoots per explant

Explants: fresh shoot stem without axillary bud

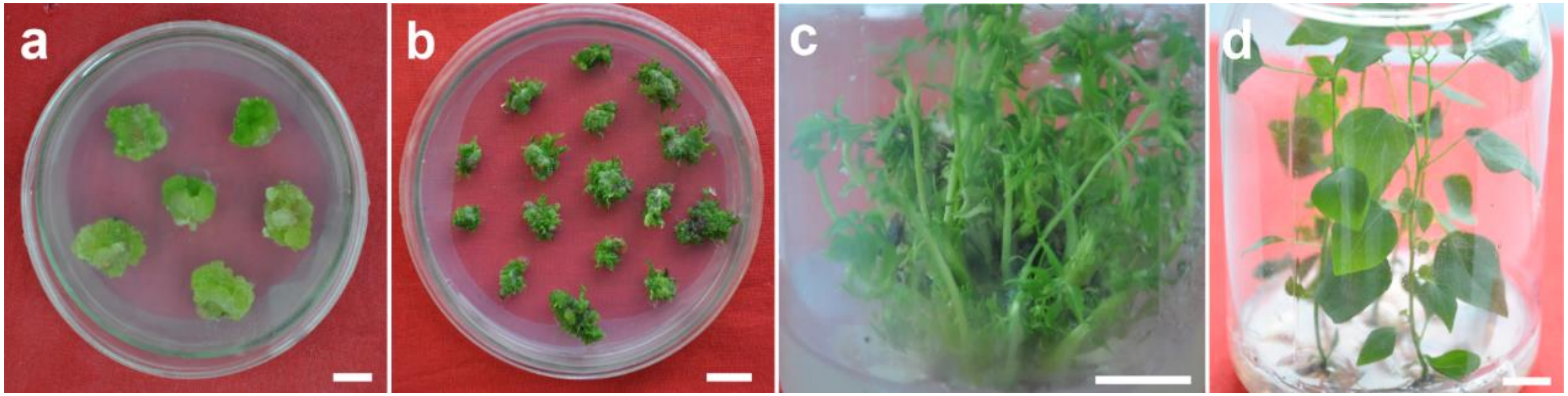


Explants: leaf disc

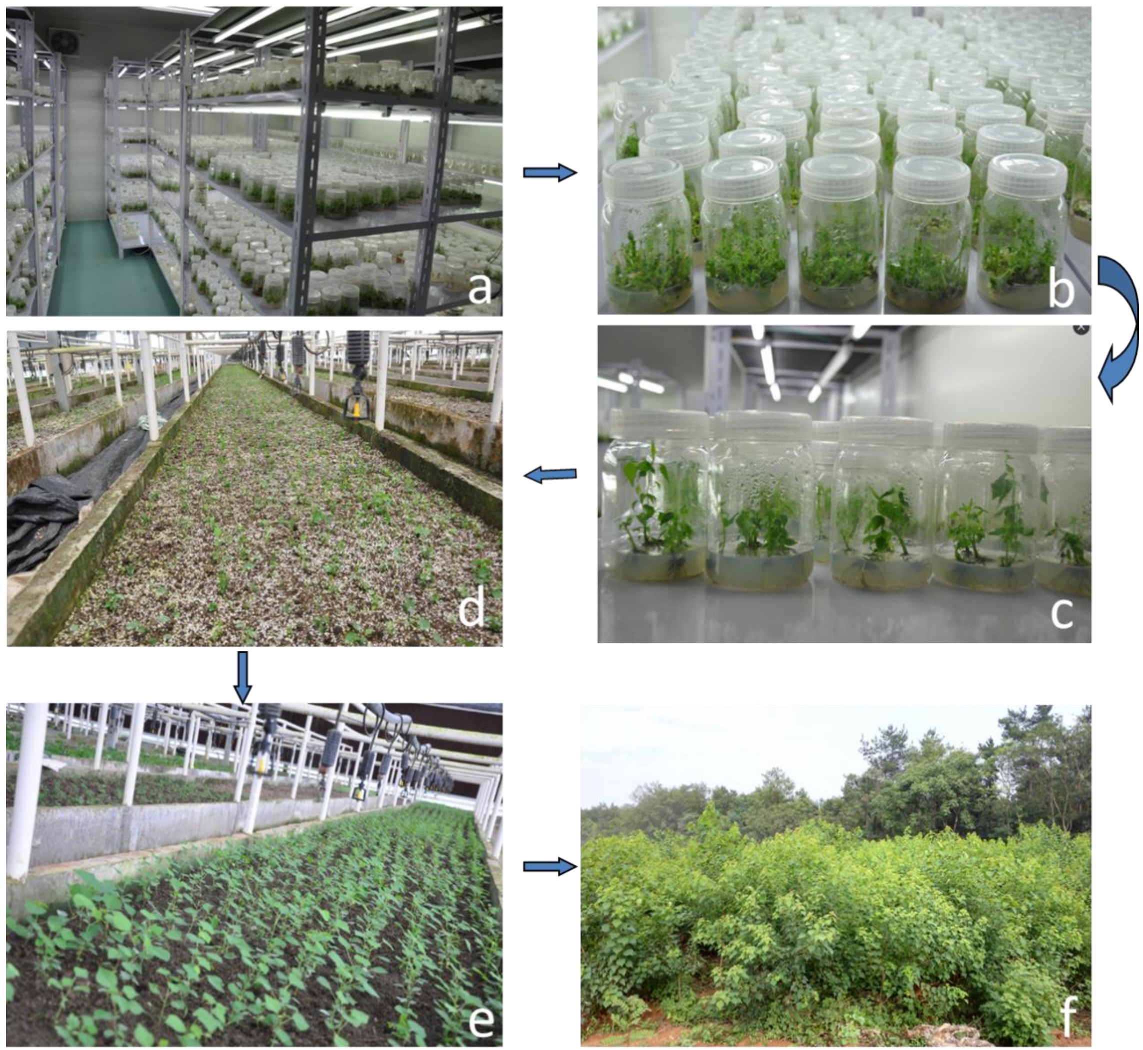


1.3 Regeneration via indirect shoot organogenesis

Explant: Shoot stem

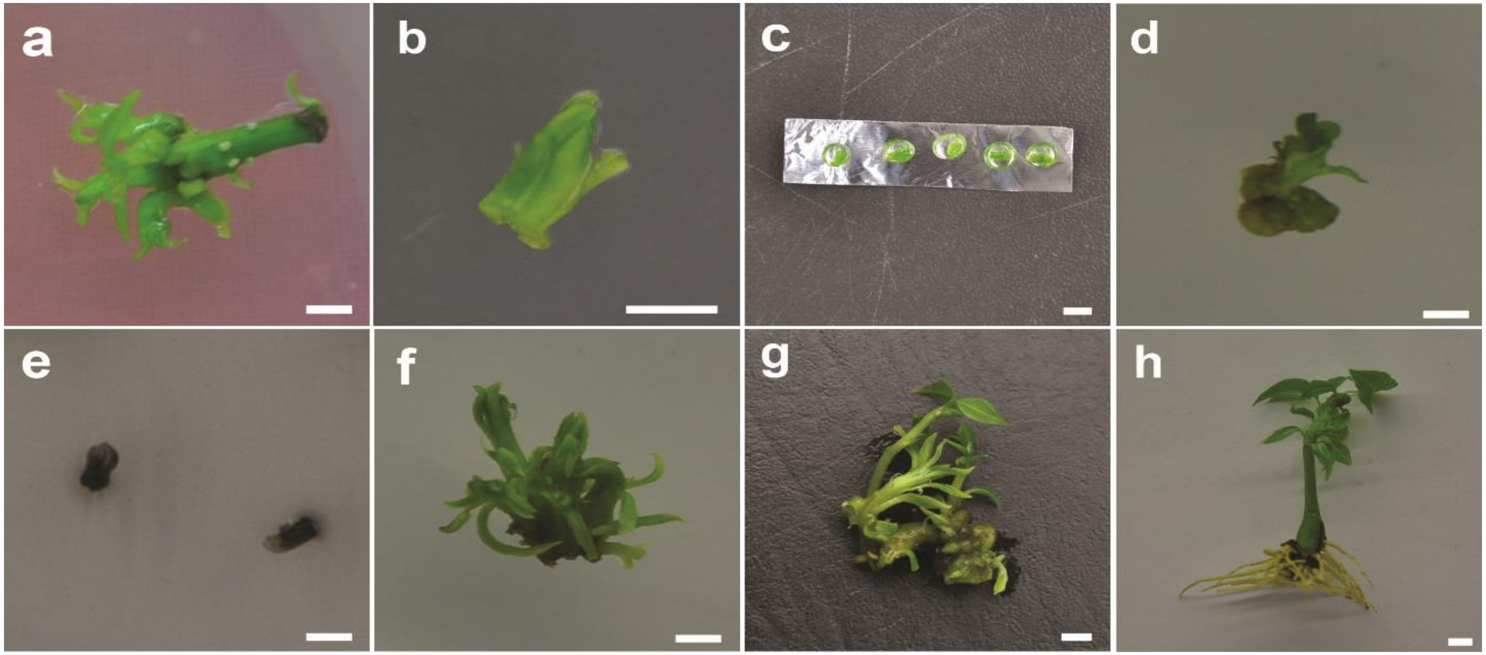


1.4 Large scale propagation of *S. sebiferum*



2. *Cryopreservation of shoot tips of S.sebiferum by droplet-vitrification*

Explant: Shoot tip

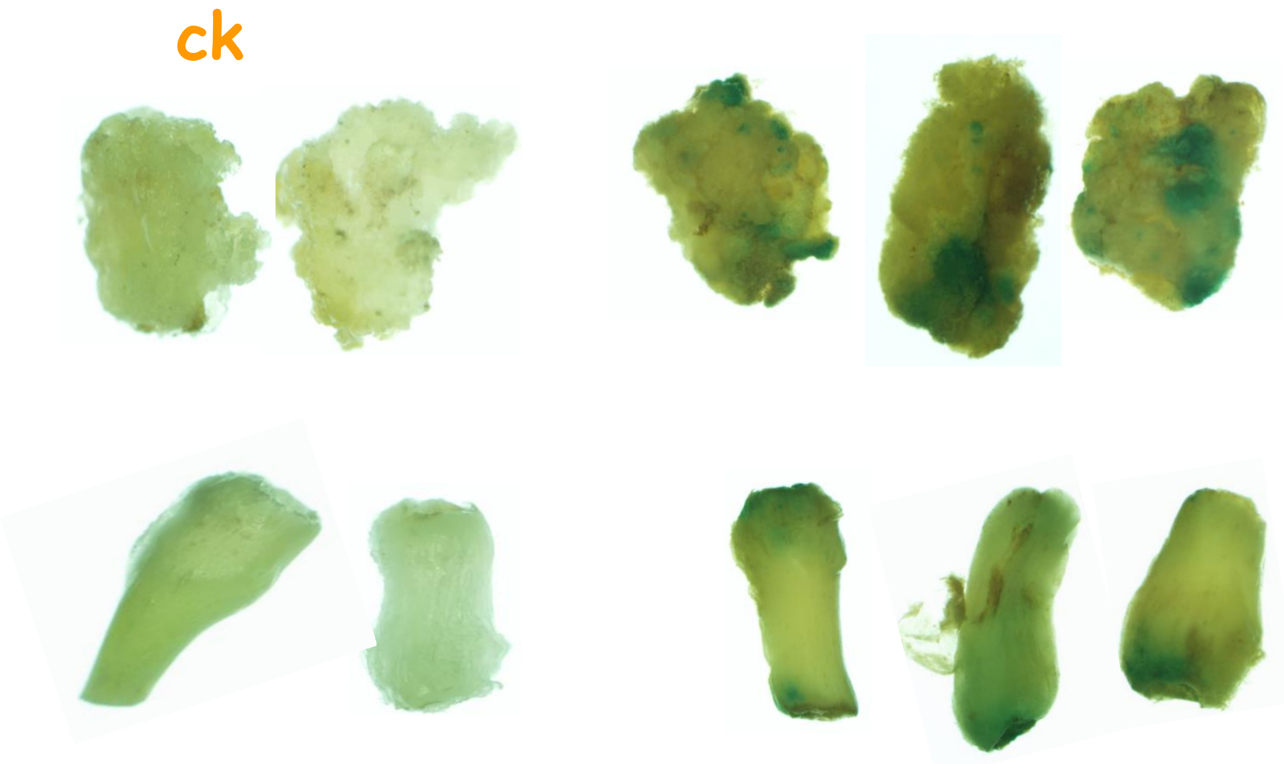


42.2% of cryopreserved shoot tips were survived and 40.0 % were regenerated

3.Agrobaterium-mediated genetic transformation

Report gene: Gus

Transient expression （93.92%）

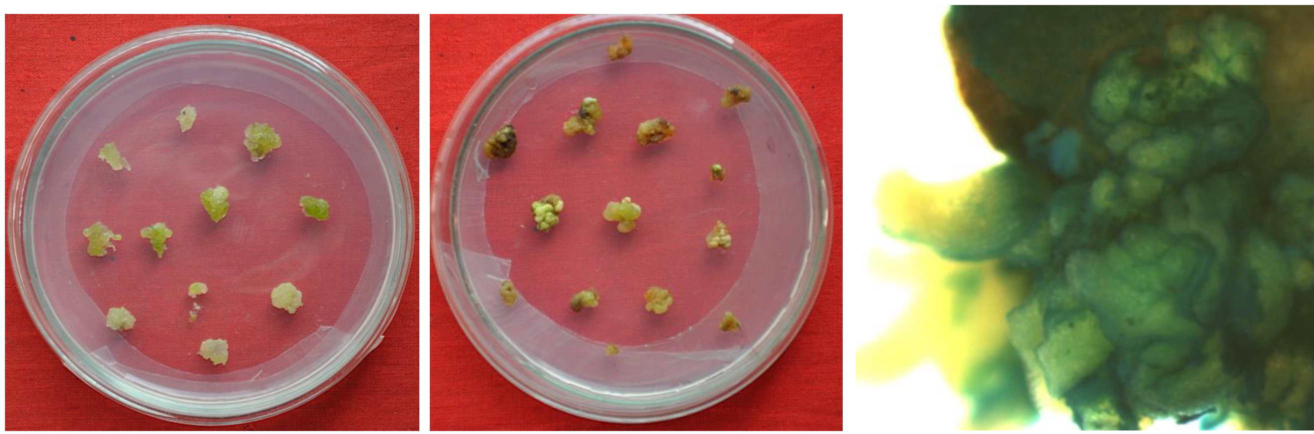


Callus

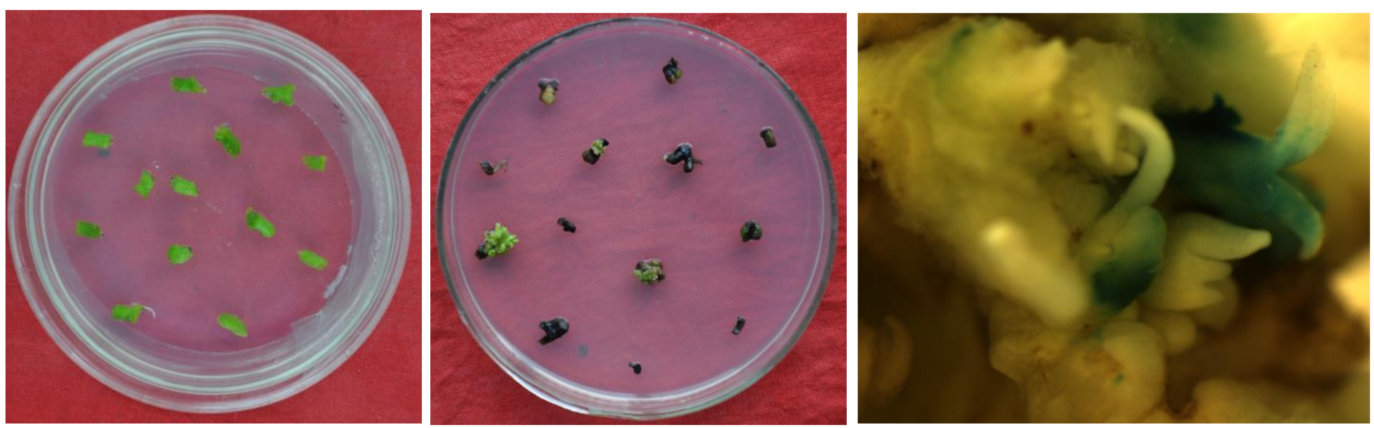
Shoot stem

Stable expression(36.7%)

Callus



Shoot stem



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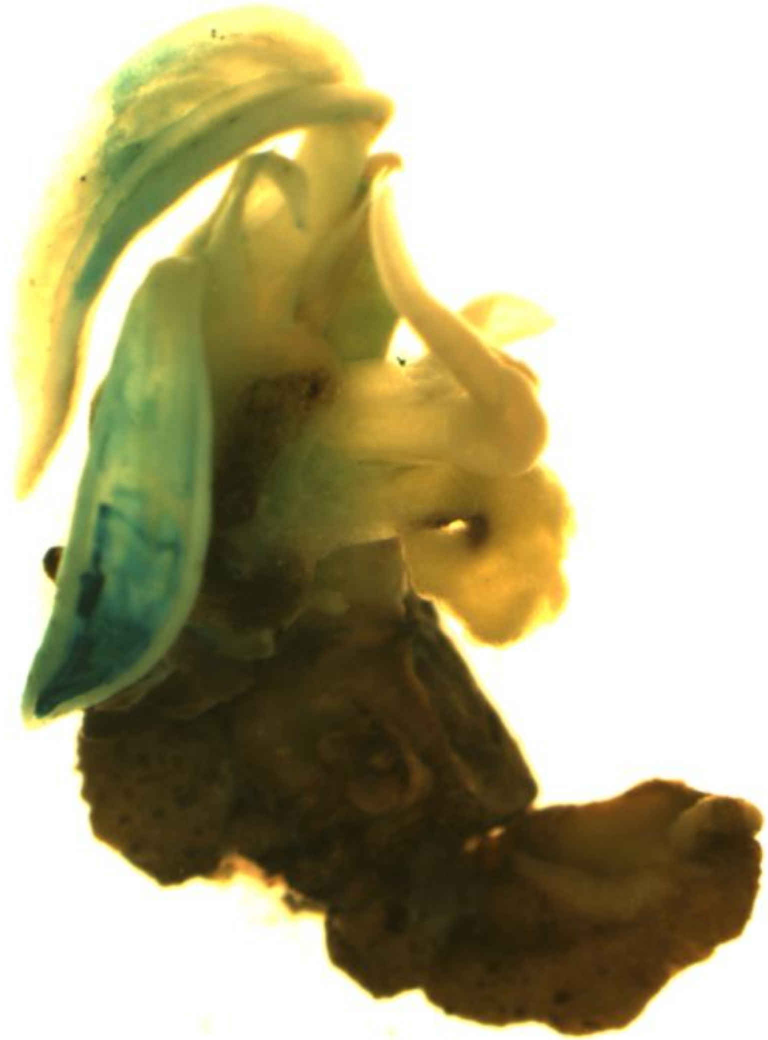
Stable expression



Stable expression

Conclusions

High efficient plant regeneration protocols were established through different pathways

Long cryopreservation system was established by droplet vitrification

Stable genetic transformation system was obtained

Thanks for your attention !