Regeneration of *Solanum nigrum* by Somatic Embryogenesis, Involving Frog Egg-Like Body, a Novel Structure

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**Abstract**

A new protocol was established for the regeneration of *Solanum nigrum* by frog egg-like bodies (FELBs), which are novel somatic embryogenesis (SE) structures induced from the root, stem, and leaf explants. The root, stem, and leaf explants (93.35%, 85.10%, and 100.00%, respectively) were induced to form special embryonic calli on Murashige and Skoog (MS) medium containing 1.0 mg/L 2,4-dichlorophenoxyacetic acid, under dark condition. Further, special embryonic calli from the root, stem, and leaf explants (86.97%, 83.30%, and 99.47%, respectively) were developed into FELBs. Plantlets from FELBs from the three explants were induced in vitro on MS medium supplemented with 5.0 mg/L 6-benzylaminopurine and 0.1 mg/L gibberellic acid, and 100.00% plantlet induction rates were noted. However, plantlet induction *in vivo* on MS medium supplemented with 20 mg/L thidiazuron showed rates of 38.63%, 15.63%, and 61.30% for the root, stem, and leaf explants, respectively, which were lower than those of the in vitro culture. Morphological and histological analyses of FELBs at different development stages revealed that they are a novel type of SE structure that developed from the mesophyll (leaf) or cortex (stem and root) cells of *S. nigrum*.

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**Introduction**

*Solanum nigrum* is a solanaceous medicinal herb commonly known as “black nightshade.” The plant has been extensively used as a traditional medicine in Asia because it contains valuable medicinal components, including glycoalkaloids (sola-nine, solamargine, solanigrine, and solasonine) [1], steroidal glycosides (β-solamargine, solasonine, and α, β-solansodamine) [2], steroidal saponins (diosgenin) [3], steroidal genin (gitiogenin) [4], and tannin and polyphenolic compounds [5]. The components can help prevent and cure liver disease [6], urinary tract infection [7], and leucorrhea [8] and promote heat clearing, detoxification [9], and dissolving stasis [10]. *S. nigrum* extracts exhibit remarkable antimicrobial activity against *Staphylococcus aureus, Typhoid bacillus, Bacillus cereus, Micrococcus kritsinae, Pseudomonas aeruginosa* [11], and *Shigella dysenteriae* [12]. In addition, its fruit is reported to have antinulcer, antioxidant, and antitumor effects in rats [13–15].

Besides its valuable medicinal components, studies on the components of its amino acids, polysaccharides, and active ingredients [16], the mechanisms of cadmium accumulation [17], somatic hybridization [18,19], anticancer mechanisms [20], herbicidal activity [21], fruit pigment [22], karyotype analysis [23], protoplast isolation [24], insecticide and virus resistance [25,26], endophytic bacteria [27], and antibacterial and antiviral abilities [28,29], have also been conducted. However, high-frequency regeneration and transformation systems in *S. nigrum* have not been established, even though low-frequency protoplast transformation with *Agrobacterium rhizogenes* [30], and direct regeneration [31], has been reported. Somatic embryogenesis (SE) offers a regeneration system with the following advantages: high propagation rates, labor saving, suitable for suspension cultures, and plantlets can be produced from genetically modified single cells. Regeneration by SE has often been used in germplasm preservation and in establishing high-efficiency transformation systems. To our knowledge, no study on SE in *S. nigrum* has been conducted. In this study, a regeneration system in *S. nigrum* was established through frog egg-like bodies (FELBs), novel SE structures, which may be used in future studies of *S. nigrum*. 

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Materials and Methods

Plant materials and explant preparation

*S. nigrum* seeds were treated with 75% (v/v) ethanol for 30 s, rinsed with sterilized distilled water three times, soaked in 2.5% (v/v) sodium hypochlorite for 8–10 min, and rinsed with sterilized distilled water five times. For germination, the sterilized seeds were sown on Murashige and Skoog (MS) medium [32] supplemented with 0.1 mg/L gibberellic acid (GA 3), 30 mg/L sucrose, and 7.8 g/L agar (pH 5.8), at 4 °C for 4 d, then incubated in a germination chamber (25 °C in the dark) until the seeds were fully germinated. Seedlings were transplanted onto MS medium at 25 °C with 16 h light (120 μmol⋅m⁻²⋅s⁻¹) and 8 h dark.

Induction of FELBs

For the optimization of supplementary plant growth regulators, the leaf, root, and stem explants were placed on MS media with 30 g/L sucrose and 7.8 g/L agar, pH 5.8, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) or 1-naphthaleneacetic acid (NAA) with the concentration series 0, 0.5, 1.0, and 1.5 mg/L. The explants were kept at 25±1 °C and in the dark to induce FELBs. The different stages of SE were recorded by using a digital single lens reflex camera (EOS 600D, Canon Inc., Japan) (Fig. 1 A, A3 and A4; B, B3 and B4; C, C3 and C4) and a stereomicroscope (SMZ800, Nikon Corporation, Japan) (Fig. 1 A1 and A2; B1 and B2; C1 and C2).

Histochemical and histological analyses of the origin and development of FELBs

For the confirmation of the presence of FELBs, double staining with acetocarmine and Evans blue [33] was used to distinguish embryonic tissue from callus. FELB embryogenic cells were stained bright red, and non-embryogenic callus was stained dark blue. The different SE stages were recorded using a Canon 600 D camera (Fig. 2 A, A1 and A4; B, B1 and B4; C, C1 and C4) or monitored using a digital fluorescence microscope (BX 61, Olympus Corporation, Japan) (Fig. 2 A2, A3, A5, and A6; B2, B3, B5, and B6; C2, C3, C5, and C6).

Staining with 4',6-diamidino-2-phenylindole (DAPI) was used to detect the nuclei of embryonic and callus cells, following a previously published method [34]. A single layer of cells was placed on a slide, and then photographed with dark-field illumination using an Olympus BX 61 microscope (Fig. 2 A3 and A6; B3 and B6; C3 and C6). Cell outlines were observed after...
using borax-toluidine blue staining; fresh material was put in borax-toluidine blue for 5 min, rinsed with distilled water five times to clean the color off, and the moisture was removed with clean filter paper. Microscopic images were recorded under light-field conditions using an optical microscope (BX 41, Olympus Corporation, Japan) (Fig. 2 A2 and A5; B2 and B5; C2 and C5).

The microscopic frozen section method developed by Y. Song (School of Life Science and Technology, Tongji University) was as follows: the materials from the different developmental stages were fixed in 4% paraformaldehyde (dissolved with 100 mM phosphate buffer solution [PBS], pH 7.2) for 48 h, dehydrated in 10% and 20% sucrose for 24 h each, and then embedded in optimum cutting temperature compound (OCT) for 2 h. The embedded tissues were then sectioned to a thickness of 8 μm with a cryostat microtome (CM1850, Leica Microsystems, Germany), and observed under an Olympus BX 41 microscope (Figs. 3, 4, and 5).

**Plantlet formation from *in vitro* and *in vivo* FELBs**

Two approaches were used for plantlet formation from FELBs. For the first approach, *in vitro* FELBs were placed on MS medium (pH 5.8) supplemented with 5.0 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L GA3. For the second approach, *in vivo* FELBs in the callus were induced into plantlets by adding different concentrations of thidiazuron (TDZ) (0, 10, 20, and 30 mg/L) in MS medium (pH 5.8). Both *in vitro* and *in vivo* FELBs were cultivated at 25°C under a 16 h photoperiod with light intensity of 120 μmol·m⁻²·s⁻¹ provided by cool-white fluorescent lights, and were subcultured monthly. Plantlets grown to 1–2 cm were separated and transferred to the rooting medium for root formation. To evaluate the frequency of regenerated plantlets from FELBs, thirty replicates of every 10 FELBs cultivated on 25 mL of medium in 90 mm petri dishes were used.
Microscopic investigation

Images of cell nuclei were taken using a digital fluorescence microscope (BX 61, Olympus Corporation, Japan) with a mirror unit (U-MNU2), a dichroic mirror (DM400), an excitation filter (BP360), and a barrier filter (BA420) for DAPI [34].

Statistical analysis

The frequencies of callus and FELB formations influenced by different plant growth regulators were analyzed using analysis of variance (ANOVA), with 99% and 95% confidence intervals, in SPSS 10.0.

Results

FELB, a novel SE structure, was induced from the leaf, root, and stem explants of *S. nigrum*

Ten days after the inoculation of the explants, obvious, translucent sticky callus (Fig. 1 A1, B1, and C1) had appeared on all three types of explant. Fifteen days after the appearance of the special calli, small white embryoid bodies had formed in the calli (Fig. 1 A2, B2, and C2). At the late developmental stage, FELBs developed into many different individual somatic embryoids, surrounded by less translucent sticky callus (Fig. 1 A3 and A4; B3 and B4; C3 and C4). In most cases, one FELB resulted in more than 10 individual somatic embryoids; sometimes one FELB could give rise to more than 40 somatic embryoids. The SE structure of the special embryogenic callus containing the somatic embryoids looked and functioned like a cluster of frog eggs, inside which the embryoids are protected and receive nutrients from the surrounding callus. Therefore, the entire SE structure was called “FELB”. To our knowledge, our study is the first to show FELBs as novel SE structures in plants.

Effects of auxin analogs on the induction of translucent sticky calli and FELBs

Two auxin analogs, NAA and 2,4-D, with a concentration series of 0, 0.5, 1.0, and 1.5 mg/L, were used to optimize plant growth regulator conditions for the induction of the translucent calli and FELBs. For all the NAA supplements with their different concentrations, no callus was induced, indicating that NAA would not be suitable for SE in *S. nigrum*. For the 2,4-D supplements, a concentration of 1.0 mg/L resulted in significantly higher rates of...
Figure 4. Microscopic images showing the development of vascular tissue in the induced embryoids of frog egg-like bodies (FELBs) and somatic embryos of *S. nigrum*. A. FELB embryoid without vascular tissue forming; B. The appearance of vascular tissue in the center of a FELB embryoid; C. Elongated FELB embryoid with developing vascular tissue; D. Vascular tissue in FELB embryoids at different developmental stages in one view; E. Longitudinal section of developed vascular tissue in a somatic embryo; F. Transverse section of a somatic embryo with developed vascular tissue; G. Developed vascular tissue in a mature somatic embryo.

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Figure 5. The regeneration process of *S. nigrum* by frog egg-like bodies (FELBs). A. Calli induced on a leaf explant; B. Enlarged view of red squares in A, showing FELBs induced in translucent sticky calli; C. Isolated FELBs; D. Isolated FELBs stained with aceticarmine and Evans blue; E. Morphologies of isolated FELBs at different developmental stages stained with aceticarmine and Evans blue; F. Morphologies of the intact individual FELBs at different developmental stages; G. The histological and morphological developmental process of FELBs; H. The regeneration process of FELBs in vitro; I, J, K, and L. The regeneration process of FELBs in vivo; M. A regenerated seedling; N. A flower from a regenerated plant; O. Immature fruits from a regenerated plant; P. Mature *S. nigrum* fruits from a regenerated plant.

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Identification and morphological analysis of FELBs

The embryogenic and callus parts of FELBs were analyzed using double staining with aceticarmine and Evans blue. The embryogenic part, in which the cells reproduce and metabolize quickly, were easily stained bright red, whereas the callus part, in which the cells could not be stained with aceticarmine because of their lower metabolism and reproduction, were detected using Evans blue (Fig. 2 A1 and A4; B1 and B4; C1 and C4). This confirmed that FELBs are composed of red embryoids, surrounded by dark blue translucent sticky calli. We suggest that a FELB is in a special SE structure during the early developmental stage of SE in S. nigrum.

The FELB embryoids were in shape similar to small red balls (Fig. 2 A1 and A4; B1 and B4; C1 and C4). By using microscopic squash technology, together with DAPI staining (Fig. 2 A3 and A6; B3 and B6; C3 and C6) and borax-toluidine blue staining (Fig. 2 A2 and A5; B2 and B5; C2 and C5), embryogenic cells of FELBs were observed to be closely arranged and with thick cytoplasm, while callus cells were much looser in arrangement than embryogenic cells (Fig. 2 A5, B5, and C5).

Histological detection demonstrated the development of vascular tissue in FELBs and mature somatic embryos

The development of vascular tissue in FELB embryoids and somatic embryos developed from FELBs were investigated using the frozen section technique. It showed that vascular tissue developed with the development of embryoids in FELBs and

| Table 1. Effect of 2,4-D on the induction of translucent sticky callus in the S. nigrum root, stem, and leaf explants. |
|---|---|---|
| 2,4-D (mg/L) | Root | Stem | Leaf |
| 0.5 | 35.60±0.55 eE | 21.73±0.54 gG | 57.20±0.50 dD |
| 1.0 | 93.33±0.54 bB | 85.10±0.36 cC | 100.00±0.00 aA |
| 1.5 | 13.47±0.35 IH | 20.63±0.59 hG | 25.77±0.33 IF |

Note: Callus induction rate refers to the ratio of explants with an induced callus to the total number of explants inoculated. The mean and standard error per treatment were calculated from 300 explants from 30 petri dishes (as 30 replicates). Capital and lowercase letters represent significant differences at the 1% and 5% probability levels, respectively. Significant differences were analyzed by the Duncan test, using SPSS 10.0.

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| Table 2. Effect of 2,4-D on frog egg-like body (FELB) induction in the S. nigrum root, stem, and leaf explants. |
|---|---|---|
| 2,4-D (mg/L) | Frequency of FELB induction (%) | |
| | Root | Stem | Leaf |
| 0.5 | 35.13±0.50 eE | 17.43±0.34 gG | 56.90±0.42 dD |
| 1.0 | 86.97±0.51 bB | 83.30±0.71 cC | 99.47±0.25 aA |
| 1.5 | 12.93±0.29 ll | 15.83±0.31 hH | 23.87±0.35 ff |

Note: Frequency of FELB induction refers to the ratio of explants with induced FELBs to the total number of explants inoculated. The mean and standard error per treatment were calculated from 300 explants from 30 petri dishes (as 30 replicates). Capital and lowercase letters represent significant differences at the 1% and 5% probability levels, respectively. Significant differences were analyzed by the Duncan test, using SPSS 10.0.

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somatic embryos (Fig. 4). The vascular tissues in FELB embryos, and the mature embryos developed from FELBs, were separate from those of the parent explants (Fig. 4), confirming that the FELBs obtained could develop into real somatic embryos.

**Plantlets could develop from in vitro and in vivo FELBs**

Two methods were used to induce plantlets from *in vitro* and *in vivo* FELBs. For plantlets developed from *in vitro* FELBs, FELBs separated from explants were transferred to the medium supplemented with 5.0 mg/L BAP and 0.1 mg/L GA₃ for the development of regenerated plantlets. The frequency of regenerated plantlets developed from *in vitro* FELBs was 100.00% (Fig 5), and individual *in vitro* FELBs often developed into more than one plantlet (Fig 5. H), which is different from the usual scenario in other plant species in which the somatic embryo often forms individually and develops into one plantlet [33,36]. For plantlets developed from *in vivo* FELBs, this study demonstrated that *in vivo* FELBs with parent callus transferred to a medium supplemented with TDZ could directly develop into plantlets, and supplemented with 20 mg/L TDZ resulted in a significantly higher frequency of the root, stem, and leaf explants than other concentrations (38.63%, 15.63%, and 61.30%, respectively; Table 3). In addition, individual *in vivo* FELBs (Fig 5. I-L) could develop into many multiple buds, and finally develop into many plantlets, as is the case for *in vitro* FELBs.

**Discussion**

The optimization of supplementation with plant growth regulators is key to the success of SE and the regeneration of plants [37,38]. In the present study, SE induction by FELBs in *S. nigrum* was achieved though supplementation with 2,4-D alone, without TDZ, whereas supplementation with NAA failed to induce FELBs. Light conditions are important for SE too; in this study, the explants being kept in the dark, instead of under moderate light as in previous studies [36,39], resulted in successful FELB induction in *S. nigrum*. Supplementing with suitable 2,4-D, and cultivating in the dark, were the prerequisites for FELB induction in this species. Under the above conditions, SE by FELBs was successfully induced from the root, stem, and leaf explants of *S. nigrum* at a high frequency. Significant differences existed in FELB induction frequencies between the three explants (leaf 99.47% > root 86.97% > stem 83.30%). All the *in vitro* FELBs, despite the origin of their explants, could be induced into plantlets. This suggests that explants from different organs of *S. nigrum* could easily be induced into somatic embryos and further plantlets under optimal conditions, but with organ differences in SE induction ability.

In most indirect SE, loose white callus is first induced and individual somatic embryos develop on the callus. In this study, translucent sticky callus was first induced and FELB, a new SE structure in *S. nigrum*, also formed. Unlike in most SE, the induced callus was translucent and sticky, and multiple embryos had been induced in the special callus. The translucent sticky callus could play a role in protecting and providing nutrients for the induced embryos in it, like the structure of a frog egg cluster. SE by FELB can be considered a new pathway for the following reasons: 1) FELB is a new type of structure not reported before; 2) the induction conditions of SE by FELBs are different from those of a typical SE pathway; 3) individual FELBs can develop into multiple plantlets, whereas usually only one somatic embryo develops into one plantlet in a typical SE pathway; 4) The special callus formed at the early stages of SE by FELB is different from that in a typical SE pathway. However, further study is needed to ascertain whether FELBs are found in other plant species.

*S. nigrum* (nightshade) is a medicinal plant with therapeutic properties belonging to the *Solanum* genus, some accessions of which exhibit a high resistance to *Phytophthora infestans*, the causal agent of potato and tomato late blight [40]. Therefore, *S. nigrum* can not only be used as medical material, but also serve as a resource of disease resistance genes for the improvement of important *Solanum* crops, such as tomato and potato. The regeneration system developed by FELBs will benefit the establishment of transformation systems in *S. nigrum*, which will be helpful for the improvement of *S. nigrum*, and its relative *Solanum* crops, and clarify the mechanism of formation of FELBs in *S. nigrum*. The developed system can be used to obtain pathogen-free plants of *S. nigrum*, because the vascular tissue of somatic embryos is independent from the parental explants [41]. In addition, the plant regeneration system developed by FELBs may give clues to establishing similar systems in other *Solanum* crops, and even the crops recalcitrant in SE induction and regeneration.

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**Author Contributions**

Conceived and designed the experiments: KX CL. Performed the experiments: KY YC KL FW ZL TZ. Analyzed the data: KX GT CL. Contributed reagents/materials/analysis tools: TL YZ FZ JZ YW WN SJ HX GT. Wrote the paper: KX CL.

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**Table 3. Frequency of plantlet induction from in vivo frog egg-like bodies (FELBs) of *S. nigrum* root, stem, and leaf explants.**

| TDZ (mg/L) | Frequency of plantlets induction (%) |
|-----------|--------------------------------------|
|           | Roots      | Stems      | Leaves     |
| 0         | 0.00±0.00 hH | 0.00±0.00 hH | 0.00±0.00 hH |
| 10        | 15.77±0.35 dD | 4.53±0.27 eE | 32.03±0.48 cC |
| 20        | 38.63±0.41 bB | 15.63±0.23 dD | 61.30±0.48 aA |
| 30        | 3.67±0.35 fF | 1.53±0.20 gG | 3.07±0.30 ff |

Note: Frequency of plantlet induction from *in vivo* FELBs of explants, as the number of induced FELBs as a proportion of the total number of explants inoculated. The mean and standard error per treatment were calculated from 300 explants from 30 petri dishes (as 30 replicates). Capital and lowercase letters represent significant differences at the 1% and 5% probability levels, respectively. Significant differences were analyzed by the Duncan test, using SPSS 10.0.

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References

1. Sun Y, Zhao Y, Wang L, Lou IX, Cheng AX (2012) Cloning and expression analysis of squameline synthase, a key enzyme involved in antifungal steroidal glycoalkaloids biosynthesis from Solanum nigrum. Drug Discov Ther 6(5):292–240.

2. Ding X, Zha FS, Li M, Gao SG (2012) Induction of apoptosis in human hepatoma SMCC-7721 cells by solamargine from Solanum nigrum L. J Ethnopharmacol 139(2):599–604.

3. Zhou X, He X, Wang G, Gao H, Zhou G, et al. (2006) Steroidal saponins from Solanum nigrum. J Nat Prod 69(8):1115–1116.

4. Sankaran M (2012) Protective effect of Solanum nigrum fruit extract on the functional status of liver and kidney against ethanol induced toxicity. J Biochem Tech 3(4):339–343.

5. Jainu M, Devi CS (2006). Antiulcerogenic and ulcer healing effects of Solanum nigrum (L) on experimental ulcer models: possible mechanism for the inhibition of acid formation. J Ethnopharmacol 104(1):156–163.

6. Javed T, Ashfaq UA, Riaz S, Rehman S, Riazuddin S (2011). In-vitro antiviral activity of Solanum nigrum against Hepatitis C Virus. Virol J 8:26.

7. Inta A, Shengji P, Balslev H, Wangpakapattanawong P, Trisonthi C (2008) A comparative study on medicinal plants used in Akha’s traditional medicine in China and Thailand, cultural coherence or ecological divergence? J Ethnopharmacol 116(3):508–517.

8. Essien EE, Ogungwande IA, Setzer WN, Ekundayo O (2012) Chemical composition, antimicrobial, and cytotoxic studies on S. eschantus and S. macroanthum essential oils. Pharma Biol 50(4):474–480.

9. Man S, Gao W, Wei C, Liu C (2012) Anticancer drugs from traditional toxic Chinese medicines. Phytother Res 26(10):1449–1465.

10. Jimoh FO, Adedapo AA, Afolayan AJ (2010) Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of Solanum nigrum and Lantana camara. Food Chem Toxicol 48(3):964–971.

11. Yogananth N, Buvaneswari S, Murhezhulian R (2012) Larcival and antibacterial activities of different solvent extracts of Solanum nigrum LINN. Global J Biotech Biochem 7(3):86–89.

12. Zubair M, Razwan K, Rassol N, Afshan N, Shadid M, et al. (2011) Antimicrobial potential of various extract and fractions of leaves of Solanum nigrum. Int J Phytomedicine 1(1):63–67.

13. Son YO, Kim J, Lim JC, Chung Y, Chung GH, et al. (2003) Ripe fruit of Solanum nigrum L. inhibits cell growth and induces apoptosis in MCF-7 cells. Food Chem Toxicol 41(10):1421–1428.

14. Prashanth Kumar V, Shashidhara S, Kumar MM, Sridhara BY (2001) Cytoprotective role of Solanum nigrum against genetaminic-induced kidney cell (Vero cells) damage in vitro. Finotera 7(2):411–416.

15. Jainu M, Devi CS (2004) Antioxidant effect of methanolic extract of Solanum nigrum berries on aspirin induced gastric mucosal injury. Indian J Clin Biochem 19(1):57–61.

16. Chen XQ, Liu Q, Jiang XY, Zeng F (2005) Microwave-assisted extraction of ribosomes from Solanum nigrum. J Cent South Univ T 12(3):536–540.

17. Mack T, Korka F, Suchova M, Skacel F, Demmerova K, et al. (1994) Accumulation of cadmium by hair-foot cultures of Solanum nigrum. Biotech Let 16(6):621–624.

18. Binding H, Jain SM, Finger J, Nordthorst G, Nehls R, et al. (1982) Somatic hybridization of an atrazine resistant biotype of Solanum nigrum with Solanum tuberosum: Part 1: Clonal variation in morphology and in atrazine sensitivity. Theor Appl Genet 63(3):273–277.

19. Zimnoch-Guzowska E, Lebecka R, Kryszczuk A, Maciejewska U, Szczerbakowa A, et al. (2003) Resistance to Pythium aphanidermatum in somatic hybrids of Solanum nigrum L. and diploid potato. Theor Appl Genet 107(1):43–48.

20. Jeong JB, Jeong HJ, Park JH, Lee SH, Lee JR, et al. (2007) Cancer-preventive peptide lanasum from Solanum nigrum L. inhibits acetylation of core histones H3 and H4 and phosphorylation of retinoblastoma protein (Rb). J Agric Food Chem 55(26):10707–10713.

21. Quakenbush LS, Andersen RN (1985) Susceptibility of five species of the Solanum nigrum complex to herbicides. Weed Sci 33:336–390.

22. Rao GR (1978) Role of fruit pigments in understanding the inter-relationship and mechanism of evolution of higher chromosomal forms of the species of the Solanum nigrum L. complex. Acta Bot Indica 6:41–47.

23. Rao MK (1971) Cytology of a pentaploid hybrid and genome analysis in Solanum nigrum L. Genetica 42(1):157–164.

24. Nehls R (1978) Isolation and regeneration of protoplasts from Solanum nigrum L. Plant Sci Lett 12(2):183–187.

25. Baghavendra K, Singh SP, Subscale barao SK, Dash AP (2009) Laboratory studies on mosquito larvicidal efficacy of aqueous & hexane extracts of dried fruit of Solanum nigrum Lin. Indian J Clin Biochem 130(1):74–77.

26. Rawani A, Ghosh A, Chandra G (2010) Mosquito larvicial activities of Solanum nigrum L. leaf extract against Culex quinquefasciatus Say. Parasitol Res 107(5):1253–1240.

27. Lao S, Wan Y, Xiao X, Guo H, Chen L, et al. (2011) Isolation and characterization of endophytic bacteria LE07 from cadmium hyperaccumulator Solanum nigrum L. and its potential for remediation. Appl Microbiol Biotechnol 89(5):1637–1644.

28. Bedford ID, Kelly A, Banks GK, Biddul RW, Cenis JL, et al. (1998) Solanum nigrum: an indigenous weed reservoir for a tomato yellow leaf curl geminivirus in southern Spain. Eur J Plant Path 104(2):221–222.

29. Venkatesan D, Karrunakaran CM, Kumar SS (2004) Studies on phytochemical constituents, functional group identification and antimicrobial activity of Solanum nigrum (Solanaceae). Bioorg Med Chem 13:2529–2532.

30. Wei ZM, Kamada H, Harada H (1986) Transformation of Solanum nigrum L. protoplasts by Agrobacterium rhizogenes. Plant Cell Rep 5(2):93–96.

31. Xu X, Yin H, Li X (2009) Protective effects of proline against cadmium toxicity in micropropagated hyperaccumulator, Solanum nigrum L. Plant Cell Rep 28(2):325–333.

32. Murashtige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15(3):473–497.

33. Gupta PK, Darzan DJ (1987) Biotechnology of somatic polymbryogenesis and plantlet regeneration in loblolly pine. Nat Biotechnol 5:147–151.

34. Xu KD, Huang XH, Wu WM, Wang Y, Chang YX, et al. (2014) A rapid, highly efficient and economical method of Agrobacterium-mediated in plants transient transformation in living onion epidermis. PLoS One 9(1):e83556.

35. Yang L, Li YH, Shen HL (2012) Somatic embryogenesis and plant regeneration from immature zygotic embryo cultures of mountain ash (Sorbus paludicola). Plant Cell Tiss Organ Cult 109:547–556.

36. Sandral C, Ana EC, Lijja S, Jorge MC (2012) Somatic embryogenesis in tamarillo (Cyttaphora betaceae): approaches to increase efficiency of embryo formation and plant development. Plant Cell Tiss Organ Cult 106(3):473–483.

37. Zhou W, Zheng S, Ling HQ (2011) An efficient regeneration system and Agrobacterium-mediated transformation of Chinese upland rice cultivar Han-dao297. Plant Cell Tiss Org Cult 106(3):473–483.

38. Zhou HC, Li M, Zhao X, Fan XC, Guo AG (2012) Plant regeneration from in vitro leaves of the peach rootstock ‘Nemaguard’ (Prunus persica var. P. davidiana). Plant Cell Tiss Org Cult 101(1):79–87.

39. Krishna KG, Dennis TT (2012) High frequency somatic embryogenesis and synthetic seed production in Citrullus lanatus. Plant Cell Tiss Org Cult 102(3):251–259.

40. Rohani ER, Ismanian I, Noor NM (2012) Somatic embryogenesis of mangosteen. Plant Cell Tiss Org Cult 110(2):251–259.

41. Lebecraa R (2008) Host-pathogen interaction between Pythium aphanidermatum and Solanum nigrum, S. tuberosum and S. selaginum. European J of Plant Path 120(3):233–240.