Supplementary Table S1. Morphological traits of regenerated plants from fresh control, non-cryopreserved and cryopreserved *Lilium* taxa

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Stock No. of GeneBank | Scientific name | Sample Status | Flower color | Stigma color | Pollen color | Floral spot | Flowering direction |
| 061001 | *L. bolanderi*  ‘Lenora’ | Freshz | Orange | Brown | Brown | Ow | Upright |
| -LNy | Orange | Brown | Brown | O | Upright |
| +LNx | Orange | Brown | Brown | O | Upright |
| 071020 | *L. callosum* var*. flavum* | Fresh | Yellow | White+Yellow | Yellow | O | Downward |
| -LN | Yellow | White+Yellow | Yellow | O | Downward |
| +LN | Yellow | White+Yellow | Yellow | O | Downward |
| 081062 | *L. bolanderi*  ‘Mount Duckling’ | Fresh | Pink | Light Brown | Light Brown | O | Upright |
| -LN | Pink | Light Brown | Light Brown | O | Upright |
| +LN | Pink | Light Brown | Light Brown | O | Upright |
| 081063 | *L. bolanderi*  ‘Mount Dragon’ | Fresh | Dark Orange | Orange | Light Brown | Xv | Downward |
| -LN | Dark Orange | Orange | Light Brown | X | Downward |
| +LN | Dark Orange | Orange | Light Brown | X | Downward |

z Fresh means *in vitro* grown without treatment

y-LN means before cryopreservation

x+LN means after cryopreservation

wO means presence of floral spot

vX means absence of floral spot

Supplementary Table S2. Experimental procedures used for cryopreservation of *Lilium* taxa.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Cold hardening | Preculture | Loading solution | Dehydration solution | Sample status | Post-culture media |
|
| Bulb-scale-bulblet (4℃) | 0.3M sucrose  (31hrs)  0.7M sucrose  (17 hrs) | LS1 (C4)  (40 min) | B1  (90 min) | -LN | PCM  (8 weeks) |
| +LN |

The bulb scales with bulblets were grown on MS medium supplemented with 0.1 mg/L IAA, 0.1 mg /L zeatin, 30g/L sucrose and 2.3 g/L phytagel was cold hardened at 4°C for 7 days in dark room. The bulblets (1.0-2.0 mm in length) were separated from the scale, prior to preculture. Precultured bulblets (MS + 0.3 M sucrose for 31 h and MS + 0.7 M sucrose for 17 h) were treated with a loading solution containing 35% of PVS3 (LS1, C4) for 40 min and exposed to dehydration solution (B1) containing PVS3 (50% glycerol + 50% sucrose) for 90 min, prior to direct immersion in liquid nitrogen (LN) for 60 min. Cryopreserved bulblets (+LN) were thawed in liquid MS medium with 0.8 M sucrose (40°C) for 10 s and transferred into liquid MS medium containing 0.8 M sucrose (RT). Thawed bulblets and non-cryopreserved bulbs (-LN) were post-cultured for regrowth in MS medium containing 3% sucrose, 15 mg/L putrescine, 0.2 mg/L zeatin, 0.15 mg/L IAA, and 0.05 mg/L GA3 (PCM medium) for 8 weeks.

Supplementary Table S3. Six ISSR markers used to analyze the genetic stability of lily accessions in this study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Primers | Sequence(5’-3’)\* | No. of amplified bands | No. of polymorphic bands | Rate of polymorphism | Range of fragment size (bp) |
| UBC814 | (CT)8A | 14 | 13 | 92.9 | 400-1700 |
| UBC 834 | (AG)8YT | 12 | 10 | 83.3 | 410-1000 |
| UBC 836 | (AG)8YA | 16 | 10 | 62.5 | 390-2000 |
| UBC 862 | (AGC)6 | 13 | 7 | 53.8 | 300-2500 |
| UBC 873 | (GACA)4 | 14 | 12 | 85.7 | 510-2000 |
| UBC 880 | (GGAGA)3 | 10 | 4 | 40.0 | 430-1400 |
| Average |  | 13 | 9 | 69.7 | - |
| Total |  | 79 | 56 | - | - |

\*Single letter abbreviations for mixed-base positions: Y = (C or T).