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The Expression of RcLEA Gene Improves Tolerance of E. coli Cells to Abiotic Stress

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Abstract [Objective] This study was to reveal the heat induced expression model of RcLEA gene and its tolerance to various abiotic stresses. [Method] Heat resistant and heat sensitive varieties of Rosa hybrida L. were subjected to heat shock treatment at 38 ℃ for 3 h, then RcLEA gene from both varieties treated was cloned and transformed into Escherichia coli strain BL21, finally recombinant colonies were separately cultured at 4 ℃ and 50 ℃ under the stresses of LiCl, NaCl, Na_2CO_3, $CdCl_2$ and H_2O_2 to study the responses of recombinant E. coli strains to high temperature, low temperature and some other abiotic stresses. [Result] After heat shock treatment at 38 ℃ for 3 h, RcLEA gene expressed highly in 'Schlossmannjein' (SM) and 'Las vegas' (LV) variety but weakly or even not expressed in 'Kordes' Perfecta' (KP), indicating that this gene is closely related with heat resistance of R. hybrida. Compared with WT strains, recombinant clones showed higher tolerance to abiotic stresses including high temperature, low temperature, heavy metal, high salt, high pH value and oxidation, suggesting that RcLEA is concerned with the response of R. hybrida to abiotic stresses mentioned above. [Conclusion] These results provide thoughts for increasing heat resistance by introducing RcLEA into heat sensitive R. hybrida varieties and studying the heat-resistant mechanism of R. hybrida, and also provide theoretical support for selecting heat resistant variety of landscape and ornamental plants like R. hybrida.
Key words Rosa hybrida L.; RcLEA; Induced expression; Abiotic stress

High temperature in summer often influences the differentiation of bud and aggravates disease and insect disasters, severely conditioning the growth and development of Rosa hybrida L. Late embryogenesis abundant (LEA) proteins are a kind of family proteins that widely exist in organisms and is related with osmotic regulation. With regard to LEA proteins, there are evidences that they are abundant in plant embryos at late growth stage, and RcLEA mRNAs could largely accumulate in plant cells under environmental stresses such as drought, low temperature, high salt, ABA, ultraviolet radiation and $NaHCO_3$[1]; moreover, in possession of high hydrophily and thermal stability, they could be in water soluble status even under boiling condition, functioning in stabilizing cell membrane, molecular barrier, ion binding and antioxidation[2]. And thereby, LEA proteins are considered as one of the protective substances to plants during stress process.

JIANG Ruiet al[3-4] extracted the soluble protein from the tender leaves of heat resistant R. hybrida variety 'Schloss mannjeim' (SM) and heat sensitive variety 'Kordes' Perfecta' (KP) both subjected to room temperature and 38 ℃ heat shock for 3 h, and by employing 2-D electrophoresis, they found specifically expressed protein points from SM after heat shock, and presumed one of which as LEA protein according to peptide mass spectrum analysis results[5]. Based upon these evidences, we cloned complete LEA cDNA sequence from R. hybrida and named as RcLEA, totally 981 bp in length, encoding 326 amino acids and with the molecular weight of 36.1. Furthermore, we studied heat induced expression model of this gene in R. hybrida via transgene, as well as the response of RcLEA to various abiotic stresses.

Materials and Methods

Experimental materials

The experimental materials are two years old potted seedlings of heat resistant R. hybrida varieties 'Schlossmannjein' (SM) and 'Las vegas' (LV), and heat sensitive variety 'Kordes' Perfecta' (KP) cultivated in China rose base of Shanghai Botanical Garden (according as the record of Shanghai Botanical Garden, data not shown). This experiment was carried out on early May, 2005.

Temperature induced expression model of RcLEA gene

Using materials under room temperature as CKs, both heat resistant R. hybrida varieties 'Schloss mannjeim' (SM) and 'Las vegas' (LV), and heat sensitive variety 'Kordes' Perfecta' (KP) were subjected to high temperature (38 ℃) for 3 h, then mRNAs from their tender leaves were extracted and purified by using RNAout kit (Tiandz Genetic Engineering), and based on which the first cDNA strand was synthesized using PrimeScript™ RT Reagent Kit (TaKaRa)[6]. Depending on 5' and 3' flanks of RcLEA ORF, forward primer 5'-ATGTCGCTTATCCCAAATTT-3' and reverse primer 5'-AAATCCAGTGTAGAGAT-3' were designed. PCR amplification program was first of 4 min at 94 ℃ for denaturation, then 30 cycles of 40 s at 94 ℃, 40 s at 55 ℃ and 60 s at 72 ℃, finally 10 min at 72 ℃ for extension.

Prokaryotic expression of RcLEA gene

Construction of prokaryotic expression vector. According to 5' and 3' flanks of RcLEA ORF, primers containing adaptors were designed by introducing EcoR I recognition site into forward primer and Sal I recognition site into reverse primer. PCR amplification was conducted as the program described above using cDNA as template. Both PCR products and pET32a were doubly digested by EcoR I and Sal I, and target fragments of PCR products and pET32a were recovered and ligated using T_4 ligase to yield recombinant construct pET32a-RcLEA. The recombinant was next transformed into Escherichia coli strain DH5α. Sequencing analysis showed that inserted RcLEA cDNA was correct and without shift frame.

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pET32a-RcLEA and pET32a (blank vector) were both transformed into *E. coli* strain BL21 and positive colonies were preserved at −80 ℃ for use.

Resistance of recombinant BL21 to temperature One milliliter of bacterial solution at OD_{600} of 1.0 generated by IPTG induction for 2 h was centrifuged at 4 500 r/min for 5 min to collect the precipitation. The precipitation was suspended with equivalent sterilized water (1.0 ml of bacterial solution at OD of 1.0 contains 1.0×10^9 cells), and 10 μl of the suspension was diluted to 1 ml, then 100 μl of the diluted bacterial solution was again diluted to 1 ml, and by analogy, diluted to the sixth round, where there are 1.0×10^3 cells in 1.0 ml solution. Next 100 μl of the six rounds diluted solution was coated on LB plates with 100 mg/L Amp and the plates were incubated at 4 ℃ for 0, 2, 4, 6, 8, 12 d under dark and subsequently transferred to 37 ℃ overnight to calculate colony number on each plate.

Meanwhile remaining bacterial solution at OD_{600} of 1.0 generated by IPTG induction for 2 h was transferred to 50 ℃ high temperature for 1, 2, 3, 4 and 5 h, and 1 ml of five bacterial solution heat shocked for different time was centrifuged to collect precipitations which were then suspended and diluted to $OD_{600}=1.0$ finally to $OD_{600}=0.01$ as described above. These solutions were coated on LB plates and incubated at 37 ℃ overnight to calculate colony number on each plate. Each experiment contained three parallel replicates.

Resistance of recombinant BL21 to other stresses Using IPTG induction culture of wild type BL21 as CK, recombinant BL21 clones harboring pET32a-RcLEA (L1, L2) and pET32a (EV) with the bacterial solution at OD_{600} of 1.0 generated by IPTG induction for 2 h was centrifuged at 4 500 r/min for 5 min to collect the precipitation. The precipitation was suspended with sterilized water to $OD_{600}=1.0$. Subsequently the solutions were coated on LB plates with different stress substances by "Z" shape inoculation using inoculating loops, cultured under dark at 37 ℃ overnight to observe colony growing state. LB plates contained five following substances for stress treatment: ① 100, 200, 300, 400 mmol/L LiCl; ② 400, 500, 550, 600 mmol/L NaCl; ③ 10, 15, 20, 25 mmol/L Na_2CO_3; ④ 300, 350, 400, 450 mmol/L $CdCl_2$; ⑤ 200, 300, 400, 500 mol/L H_2O_2.

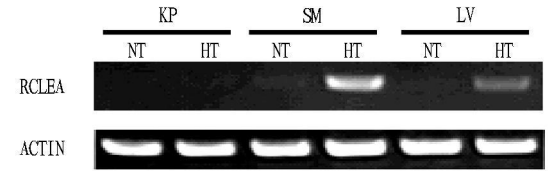
Results and Analysis

Temperature induced expression mode of RcLEA gene

As indicated from RT-PCR amplification results (Fig. 1), RcLEA gene did not express in all *R. hybrida* varieties tested under room temperature (25 ℃), while highly expressed in heat resistant varieties SM and LA, and poorly in heat sensitive variety KP under heat shock conditions (38 ℃ for 3 h), suggesting that RcLEA gene expression in *R. hybrida* varieties is heat inducible.

Resistance of recombinants to high temperature and low temperature

Under high temperature (50 ℃), colony number of recombinant clones sharply decreased with the treatment duration lasting. Contrastively, recombinants harboring plasmid pET32a-RcLEA performed high resistance to high temperature, whose survival rate reached 70.9% after 1 h heat shock treatment, and still 4.5% after 5 h, while survival rate of those harboring blank vector pET32a decreased to 11.9% after 1 h heat shock treatment, and to zero after 4 h (Fig. 2).



NT: Normal temperature; HT: High temperature; KP: Kordes' Perfecta; SM: Schpss mannejm; LV: Las vegas.

Fig. 1 RT-PCR results for expression of RcLEA in *R. hybrida* varieties under different temperature treatment

This shows that expression of RcLEA has remarkably enhanced the resistance of recombinants to high temperature.

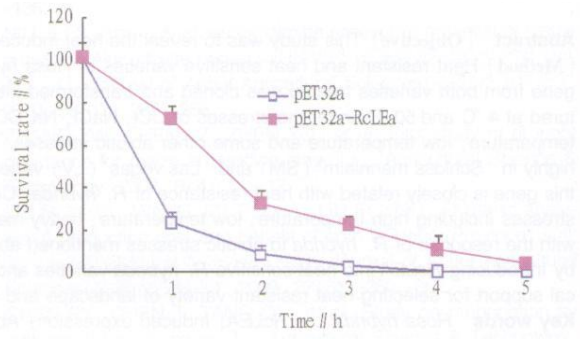


Fig. 2 Viability assay of *E. coli* strain BL21 with or without pET32a-RcLEA under 50 ℃

As shown in Fig. 3, colony number of recombinants either harboring pET32a-RcLEA or pET32a decreased gradually with the low temperature stress (4 ℃) lasting, while those harboring pET32a-RcLEA decreased obviously slower than harboring blank vector pET32a. After low temperature stress for 12 h, colony number of the latter decreased to 33%, while of the former still kept at 65%, indicating that expression of RcLEA has remarkably enhanced the resistance of recombinants to low temperature.

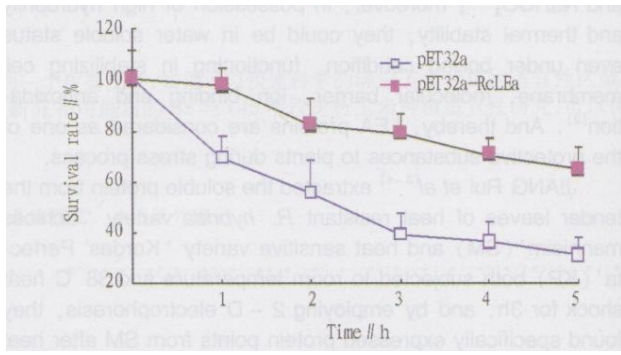


Fig. 3 Viability assay of *E. coli* strain BL21 with or without pET32a-RcLEA under 4 ℃

Resistance of recombinants to other abiotic stresses

For WT and both recombinants harboring pET32a or pET32a-RcLEA (two replicates of L1 and L2), low concentration of LiCl showed no obvious inhibitory effect; when LiCl concentration increased to 300 mmol/L, those harboring pET32a-RcLEA (L1 and L2) showed growth rates of about 50%, while harboring WT and EV of lower than 10%. $CdCl_2$ at the concentration of 350 mg/L heavily inhibited the growth of WT and EV (showing the growth rates of about 10%), while slightly to L1 and L2 (about 50%) whose colonies could grew to the verge of plates through the number was less. Results of both above experiments suggest that expression of RcLEA gene confers *E. coli* the resistance to heavy metals.

Stressed by 550 mmol/L NaCl, both L1 and L2 could grew about 65% colonies, while WT and EV were heavily restricted, indicating that resistance of recombinants to high salt increased. Toward 15 mmol/L of Na_2CO_3, L1 and L2 could grow to the verge of plates (about 40% of CKs), while WT and EV were obviously restricted (lower than 10% of CKs), suggesting recombinants performed higher resistance to high pH value. H_2O_2 at the concentration of 400 mol/L inhibited four recombinants to different extent detailedly L1 and L2 could grow to the verge of plates (about 45% of CKs), while WT and EV just assumed less colonies surrounding the streaks (about 10% of CKs), which manifests that recombinants expressing RcLEA are endowed with higher resistance to oxidation stress.

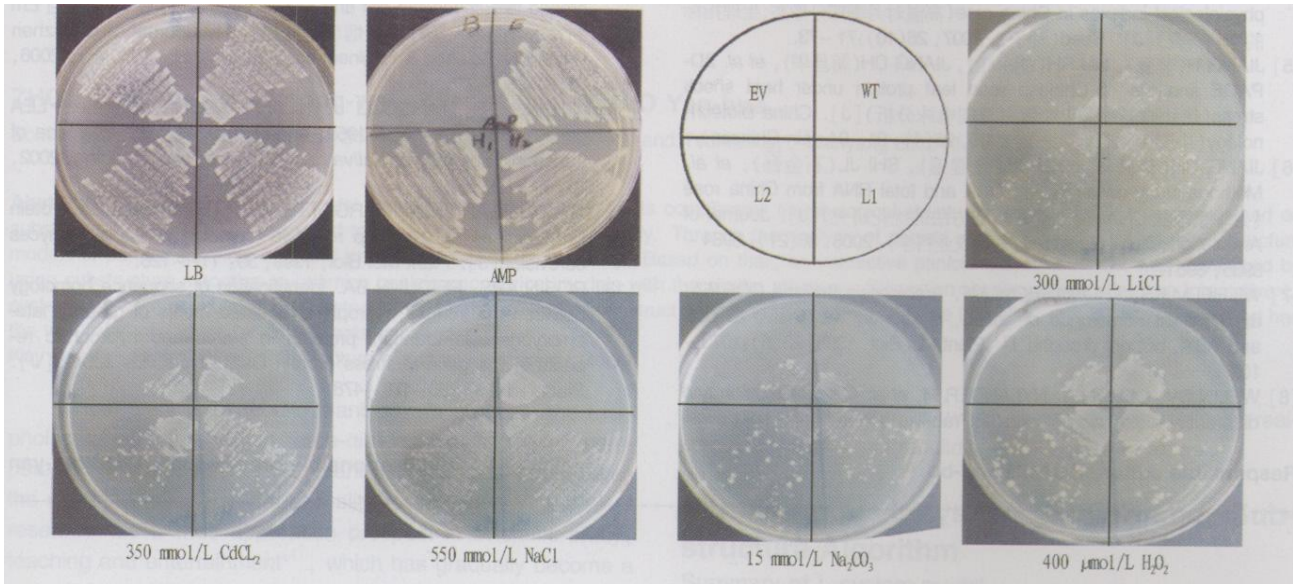


Fig. 4 Viability assay of E. coli strain BL21 under various stresses

As described above, RcLEA fusion protein accumulate largely in recombinants with pET32a-RcLEA, remarkably enhancing the resistance of host E. coli strain BL21 to abiotic stresses including heavy metal, high salt, high pH value and oxidation. In conclusion, RcLEA protein could response to various abiotic stresses.

Discussion

Expression of some Arabidopsis LEA genes is constitutive under normal condition, while some others are highly inducible under the stresses of drought, cold and high salt[7-8], which is confirmed in R. hybrida in the present study. RcLEA gene showed an expression model of heat shock inducible under high temperature stress, but it just highly expressed in heat resistant varieties and poorly or even not expressed in heat sensitive R. hybrida varieties. Whether the expression of RcLEA gene is inducible answers to the heat resistance of R. hybrida variety observed in the field[3-4,9].

Soybean LEA encoding gene Em has been proved to be able to improve salt resistance of E. coli and tobacco plants[10]. Since first time discovered from cotton cotyledon in 1981 by Dure et al, LEA proteins have been found highly express in seeds, anther and nutritional tissues of drought stressed seedlings of various plants, and its expression level is closely related with adversity resistance of cells. Up to the present, there are many literatures focusing the function of LEA to plants under stress. Cheng et al[11] introduced wheat PMA1959 (LEA1) gene into rice and found transgenic rice plants are provided with higher capacity to dehydrate and high salt using the similarity of yeast cell and plant cell in response to high salt. Swire-Clark transformed wheat Cm gene into yeast and found that single LEA protein directly improve the resistance of recombined yeast to high concentration of NaCl, KCl and low temperature stress[12]. By introducing soybean Em gene (LEA1) into E. coli and tobacco, CAID an et al[10] proved that over expression of this gene not only directly contributes to the improvement of recombinants' salt resistance, but also to the enhancement of tobacco plants' resistance to high salt. Their results provided evidence for former researchers who studied this mechanism in rice and yeast and put forward the hypothesis, i.e., LEA proteins in both prokaryotic and eukaryotic cells may adopt similar protective mechanism for adversity resistance[13]; moreover the results of CAID an et al also confirmed that E. coli heterogenous expression is the simple, shortcut and effective system to study the salt resistance mechanism of LEA protein. To validate the functions of RcLEA in R. hybrida, we cloned it and transformed into E. coli strain BL21, and revealed RcLEA gene conferred high resistance to high temperature and low temperature, as well as to other abiotic stresses like high salt, high pH value, heavy metals and oxidation. Because E. coli itself can not synthesize LEA protein under heat shock, improvement of E. coli in the resistance to various stresses is regarded as being directly related with the expression of RcLEA gene from R. hybrida. The results indicate that R. hybrida RcLEA gene plays a role in the response to various abiotic stresses, which provides thoughts for increasing heat resistance by introducing RcLEA into heat sensitive R. hybrida varieties and studying the heat resistant mechanism of R. hybrida, and also provides theoretical support for selecting heat resistant variety of landscape and ornamental plants like R. hybrida.

References

[1] THOMASHOW MF. PLANT COLD ACCLIMATION: freezing tolerance genes and regulatory mechanisms[J]. Annu Rev Plant Physiol Plant Mol Biol, 1999, 50: 571-599.

[2] BAKER J, DENNSTEELE C, DARE L. Sequence and character-

ization of 6 Lea proteins and their genes from cotton[J]. Plant Molecular Biology, 1988, 11(3): 277-291.
[3] JIANG CH(蒋昌华), HU RH(胡永红), QIN J(秦俊), et al. Research in effect of high temperature on physiological indexes of varieties in China rose (高温胁迫对月季品种部分生理指标的影响研究)[J]. Seed(种子), 2008, 27(6): 31-34, 38.
[4] HU RH(胡永红), JIANG CH(蒋昌华), QIN J(秦俊), et al. Research for effects of high temperature on several morphological physiological indexes in China rose(高温对月季部分形态、生理指标的影响研究)[J]. Seed(种子), 2007, 26(10): 71-73.
[5] JIANG R(姜蕊), HU RH(胡永红), JIANG CH(蒋昌华), et al. 2D-PAGE analysis of Chinese rose leaf protein under heat shock stress(热胁迫下月季叶片蛋白双向电泳分析)[J]. China Biotechnology(中国生物工程杂志), 2006, 26(4): 91-94. (in Chinese)
[6] JIANG CH(蒋昌华), XU JY(徐健遥), SHI JL(石金磊), et al. Methods study on extracting DNA and total RNA from China rose (月季基因组 DNA与总 RNA提取方法的优化研究)[J]. Journal of Anhui Agricultural Sciences(安徽农业科学), 2008, 36(21): 8934-8935, 8951.
[7] WILHELM KS, THOMASHOW MF. Arabidopsis thaliana COR15b, an apparent homologue of cor15a, is strongly responsive to cold and ABA, but not drought[J]. Plant Mol Biol, 1993, 23(5): 1073-1077.
[8] WELIN BV, OLSON A, NYLANDER M, et al. Characterization and differential expression of dhn/lea/rab-like genes during cold acclimation and drought stress in Arabidopsis thaliana[J]. Plant Mol Biol, 1994, 26(1): 131-144.
[9] JIANG CH, XU JY, ZHANG H, et al. A cytosolic class I small heat shock protein, RcHSP17.8, of Rosa chinensis confers resistance to a variety of stresses to Escherichia coli, yeast and Arabidopsis thaliana[J]. Plant Cell and Environment, 2009, 32: 1046-1059.
[10] CAI D(蔡丹), ZHENG YZ(郑易之), LAN Y(兰英). Expression of Em gene (LEA1) from soybean immature seeds confers salt tolerance to Escherichia coli and tobacco plants(大豆 LEA蛋白 Em的表达可提高大肠杆菌和烟草耐盐性)[J]. Journal of Shenzhen University Science & Engineering(深圳大学学报, 理工版), 2006, 23(3): 230-236.
[11] CHENG ZQ, TARGOLLI J, HUANG XQ, et al. Wheat LEA genes, PMA80 and PMA1959, enhance dehydration tolerance of transgenic rice (Oryza sativa L.)[J]. Molecular Breeding, 2002, 10: 71-82.
[12] SWIRE-CLARK GA, MARCOTTE WR. The wheat LEA protein Em functions as an osmoprotective molecule in Saccharomyces cerevisiae[J]. Plant Mol Biol, 1999, 39: 117-128.
[13] ROBINAPS, REDDY BA. Identification of sequence homology between the internal hydrophilic repeated motifs of group 1 late-embryogenesis-abundant proteins in plants and hydrophilic repeats of the general stress protein GsiB of Bacillus subtilis[J]. Planta, 1998, 206: 476-478.

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月季 RcLEA基因表达提高大肠杆菌对非生物胁迫耐性(摘要)

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[目的]研究 RcLEA基因在月季品种中热诱导表达模式及 RcLEA基因对多种非生物胁迫耐性。
[方法]将耐热和不耐热月季品种进行 38 ℃/3 h热激处理, 研究 RCLEA基因在月季品种中的热诱导。表达模式; 为验证月季 RcLEA基因功能, 将其转化 E. coli BL21, 将重组菌株 BL21分别置于 4 ℃、50 ℃及 LiCl、NaCl、Na_2CO_3、CdCl、H_2O_2胁迫下, 研究重组菌株对高温、低温及非生物胁迫的响应。
[结果]38 ℃/3 h热激处理后, 该基因在耐热月季品种‘曼海姆宫殿’(Schlossmannjeim, SM)、‘赌城’(Las vegas, LV)中强表达, 而在不耐热品种‘新十全’(Kordes'Perfecta, KP)弱表达或不表达, 表明该基因与月季耐热性关系密切。重组菌株提高了寄主大肠杆菌对高温、低温、重金属、高盐、高 PH值、氧化等非生物胁迫的耐性, 表明 RcLEA参与了上述非生物胁迫的响应。
[结论]该研究为后续该基因导入不耐热月季品种提高月季耐热品质及其机理研究提供了思路, 也为月季等园林观赏植物的耐热品种筛选提供了理论支持。
关键词 月季; RcLEA基因; 诱导表达; 非生物胁迫

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