Advanced Studies in Biology, Vol. 13, 2021, no. 1, 29 – 36
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2021.91295

Integration of Traditional and Molecular Breeding to Improve Drought Tolerance of Groundnut

D. T. K. Cuc, P. T. Phuong, N. T. T. Loan, N. D. Cuong, N. T. Ngoan, N. L. Phuc, D. T. C. Anh, T. D. Khanh, K. H. Trung*

Agricultural Genetics Institute, Hanoi Vietnam
*Corresponding author

Abstract

Groundnut (*Arachis hypogaea* L.) is an industrial crop, short-term food crops with high economic and nutritional value. In Vietnam, groundnuts play an important role in crop restructuring programs, especially in areas where the climate is constantly changing and farming conditions. In this study, we attempted to improve the drought tolerance ability of groundnut by traditional and molecular breeding methods. The results showed that the LCH-01 selected by both phenotyping and genotyping from BC_2F_3 of the combination of the "Sen That" and "L18" (recipient and donor parents), has had good agronomic traits and higher drought tolerance ability under the water deficit treatment. The selected line LCH-01 showed good drought tolerance in the artificial drought condition. This line was then confirmed by using molecular markers, 3 SSR markers were polymorphic and possibly linked to the drought tolerance gene/QTLs and may be responsible for the drought tolerance ability of this line. This line is ongoing to be recognized as the national peanut variety and will be released to the drought peanut growing areas in the coming time.

Keywords: Groundnut, drought tolerance, molecular marker

Introduction

Groundnut (*Arachis hypogaea* L.) is an industrial crop, short-term food crops with high economic and nutritional value [1]. Increasing demand for groundnut consumption has encouraged many countries to invest and develop groundnut on an expanding scale. Groundnut is one of the food sources of fat and

protein necessary for the human diet. Besides, groundnuts also contain B vitamins and a certain amount of carbohydrates. In Vietnam, groundnuts play an important role in the restructure of crops, especially in areas where the climate is constantly changing and farming conditions still face many difficulties [2]. In recent years, the review of practical experience and the application of advanced science in production have contributed to a significant increase in groundnut productivity. However, the level of productivity increase depends on the climatic conditions and the farming regime of each region. For instance, Bac Giang is one of the provinces with a relatively large groundnut growing area in Vietnam. On the poor soil in the mountainous midlands with high bare feet, the farming conditions depend mainly on the rainfall. Most of the land area for short-term industrial crops such as groundnut is limited. As a solution, it is necessary to select and create new groundnut varieties with drought tolerance, high yield, and adapt to ecological zones where inactively irrigating. The molecular breeding method was used to shorten breeding and selecting time to overcome the weakness of traditional methods.

In this study, our attempts have made to improve the drought tolerance ability of groundnut by traditional and molecular breeding, we have also focused on evaluating the drought tolerance ability to verify the inheritance of drought tolerance alleles of "Sen That" landrace germplasm of domestic groundnut variety to LCH – 01.

Materials and Methods

Materials

"Sen that" domestic groundnut variety which contains drought tolerance traits but has low productivity and was used as a donor, while L18 imported groundnut variety with high productivity, weak response under drought stress conditions was used as a recipient.

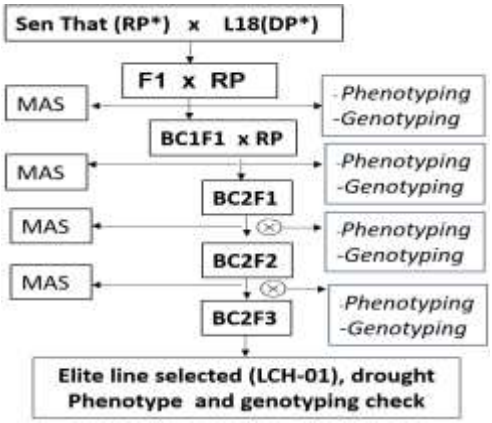

Figure 1: A breeding scheme to generate "LCH-01" line

"Sen that" and L18 and "LCH-01" were provided by Legume Research and Development Center, Agriculture Genetics Institute (AGI). "LCH-01": Offspring of "Sen that" x L18 crossing combination which was selected and crossed by High-tech Biology Experimental Center collaborated with Legume Research and Development Center (Figure 1.).

Methods

DNA extraction, PCR, gel electrophoresis and molecular application

The leaf samples were collected from a 2-week old plant which had been grown in net house of Agricultural Genetics Institute. DNA was extracted from leaf samples by using CTAB method of Genetics laboratory, Ghent University, Belgium). The PCR reaction and gel electrophoresis was generated on Eppendorf Mastercycle EP gradient 96 wells by using plate 0.2ml 96 wells provided by Biologix and following the method of Cuc et al [3].

For the marker application, a total 9 SSR markers involved in the drought tolerance gene/QTLs and were previously reported [4, 5, 6] were selected and shown in Table 1.

Table 1. The information of markers related to the target genes/QTLs of drought tolerance were used in this study

No	Marker	Forward & reverse sequences (5'-3')	SSR motif	Band size bp	AT°C
1.	TC1A02	Fw: GCAATTTGCACATTATCCGA Rv: CATGTTCGGTTTCAAGTCTCAA	(TC)35	240-276	59
2.	TC3A12	Fw: GCCCATATCAAGCTCCAAAA Rv: TAGCCAGCGAAGGACTCAAT	(TC)27	202–240	59
3.	TC3H07	Fw: CAATGGGAGGCAAATCAAGT Rv: GCCAAATGGTTCCTTCTCAA	(GA)20+(TTC)4	246-270	59
4.	TC4D02	Fw: AAGTTGTTCCCGTTGCACTC Rv: AAAACACCATAAGGTGAATCAAA	(CT)21	166-220	59
5.	TC4E10	Fw: ACGTCATCTTCCCTCCTCCT Rv: CCATTTTCTCCTCGAACCAA	(CCT)4 + (T)12 + (CT)14	300-344	59
6.	TC9F10	Fw: ATCACAATCACAGCTCCAACAA Rv: GGCAAGTCTAATCTCCTTTCCA	(AG)31	286-320	59
7.	TC11A04	Fw: ACTCTGCATGGATGGCTACAG Rv: CATGTTCGGTTTCAAGTCTCAA	(CT)16 + (CT)33	172-204	59
8.	RI2D06	Fw: AACACCTCAAATTCCCTATCCT Rv: AAAAACGCGCTGGAGTTC	(TCC)5	275-290	59
9.	RN0x614	Fw:CAGAACAAGCCACAACAAGAAG Rv: TTCAAGTCCAAGCACCTAACC	(CAA)7	220-241	59

Evaluating drought tolerance under open conditions

L18, "Sen that" and "LCH-01" had been grown in a pod (Diameter: 40cm; Height: 50cm), each pod contained 10kg alluvial soil. The soil was dried, ground and mixed with basic fertilizers: 0.75g Urea, 5.6g Phosphate, 1.5g Potassium/Pod. Each pod had 8 seeds, watering until the humidity of soil reached 70-80%. When the plant developed and had two leaves, cut the plants in each pod to remain 4 plants/Pod. All growing pods had been kept in the net house and covered with nylon film. Temperature and air humidity depended on environmental conditions.

The impact of drought would be assessed in 2 periods: (i) Flowering period, (ii) Fruit formation period. Therein, each assessment period would have 2 experiments: (1). Watering equally during the growth and development of a plant to maintain the soil humidity in a range of 70-80%. (2). Watering to keep the soil humidity maintained around 70-80% until the beginning of each assessment period then stopped watering and kept the plants withered (completely). At the time when the groundnut plants were dried-up, took the plant and soil samples to evaluate the drought tolerance ability of each variety.

The drought tolerance ability under artificial conditions based on the wilted and recovered levels of the plant. In this experiment with a similar design, the evaluation would be conducted in 2 periods: (i) Flowering period, (ii) Fruit formation period, stopped watering and kept dry in 10 days for each period, observed and assessed the dried grade and recovered levels through the leaves of plants. Assessing groundnut under drought stress condition based on Permanent Wilting Point (PWP) and the height of the main stem with some minor modification [7, 8]:

$$PWP\ (\%) = M1 - M2M2 *100\%$$

With: M1 is the weight of the soil in a permanent wilting point before drying.

M2 is the weight of the soil in a permanent wilting point after drying.

- Height of main stem: Measuring from the base of the stem to the newest leaf.
- Evaluating the drought tolerance ability under artificial conditions through wilted level and recovered level of plant-based on the criteria:

Wilted level: level 1 = 10 - 20% ; level 2 = 20 - 40%; level 3 = 40 - 60% ; level 4 = 60 - 80%; level 5 = 80 - 100% wilted leaves.

Recovered level: Level 1 = completely recovered (100% leaves recovered); level 2 = medium recovered (>60% leaves recovered); level 3 = modest recovered (< 50% leaves recovered).

Statistical Analyses

All data were done by using Excel version 2016, and IRRISTAT 5.0 for statistical analysis.

Results and Discussion

The evaluation of drought tolerance ability of LCH-01 groundnut line

To assess the drought tolerance and the development of original parents and the “LCH-01” backcrossed line, we did the comparison between the height of the main stem under drought stress condition and control condition, wilted humidity of three groundnut lines during the flowering stage.

After growing and measuring the height and calculating the wilting humidity, the data and the evaluation of drought tolerance ability were summarized in table 1:

Table 1. The assessment of groundnut varieties during the flowering stage

Groundnut variety/line	The height of main stem (cm)		Wilting humidity(%)	Drought tolerance ability
	Drought treatment	Control		
L18	14.3 ± 0.5	11.2 ± 0.2	8.38	Weak
Sen That	15.7 ± 0.3	14.2 ± 0.1	4.29	High
LCH-01	17.4 ± 0.1	16.4 ± 0.1	4.47	High

Following the data of table 2, the LCH-01 backcrossed line had the length of the stem higher than the parental line and maternal line (increased about 10.83% compared to Sen That and 21.68% higher than L18; while the data for control treatment showed that LCH-1 was 46.43%, 15.49% higher than L18 and Sen That, respectively.). Otherwise, the L18 groundnut line less stable with the standard deviation was 0.5 cm in drought treatment and 0.2 cm in the control treatment, compared to Sen That (0.3 cm in drought treatment, 0.1 cm in control treatment), especially LCH-01 (0.1 cm in drought treatment, 0.1 cm in control treatment).

Besides, the wilting humidity of L18 was highest, which was 8.38%, higher than Sen That and LCH-01 which were 4.29%, 4.47% respectively. As a result, L18 had the worst drought tolerance ability compared to the others based on wilted humidity.

Assessment of the drought tolerance ability under artificial conditions through the wilted level and recovered level of groundnut plants

To evaluate the drought tolerance ability in detail, we measured the wilted level and recovered the level of groundnut lines in 10 days after drought treatment, 3 days irrigating after drought treatment, respectively during the flowering and seeding stage.

Table 2. The affection of drought stress condition on wilted level and recovered level of groundnut lines

Name	Flowering stage		Fruit formation stage	
	Wilted level in 10 days after drought treatment (1-5)	Recovered level in 3 days irrigated back after drought treatment (1-3)	Wilted level in 10 days after drought treatment (1-5)	Recovered level in 3 days irrigated back after drought treatment (1-3)
L18	5	2	5	3
Sen That	3	1	5	2
LCH-01	3	1	4	2

Wilted level: level 1 = 10 - 20% ; level 2 = 20 - 40%; level 3 = 40 - 60% ; level 4 = 60 - 80%; level 5 = 80 - 100% wilted leaves.Recovered level: Level 1 = completely recovered (100% leaves recovered); level 2 = medium recovered (>60% leaves recovered); level 3 = modest recovered (< 50% leaves recovered).

As a result of table 2, during the flowering stage, the wilted level of L18 in 10 days after drought treatment which was in level 5 – 80%-100% leaves had wilted while Sen That and LCH-01 were only in level 3 – 40%-60 wilted leaves.

Furthermore, the recovered level of L18 also the lowest level – level 2 which had only more than half of leaves could recover. But the data for Sen That and LCH-01 were similar which had level 1- completely recovered.

In the fruit formation stage, the wilted level in 10 days after drought treatment of L18 equal to the wilted level of Sen That (level 5 – 80% - 100% wilted leaves). However, the LCH-01 only had 60%-80% wilted leaves which were level 4, the lowest level in the groundnut lines. The recovered level of LCH-01 and Sen That in the seeding stage were level 2 - > 60% leaves recovered while the data for L18 was in level 3 – less than 50% leaves could recover.

In general, the LCH – 01 backcrossed and selfing line which was generated by the cross combination between L18 and Sen That had a good drought tolerance ability compared to L18 and Sen That. However, the LCH – 01 had several criteria better than Sen That which good in drought tolerance such as wilted level in 10 days after drought treatment (1-5) during seeding stage (level 4 in LCH-01 and level 5 in Sen That) the height of the main stem.

Identification of SSR marker linked to drought tolerance traits in "LCH-01" backcrossed line and parent lines

Our efforts were to find out the SSR markers linked to drought tolerance traits for three DNA samples. Of which, Sen That, L18, the recipient and donor parents, and the offspring LCH – 01 generated from BC2F3. With the SSR markers which had expected product about 300bp, we did the electrophoresis on gel agarose 3%, while the SSR markers had expected lower than 300bp run in polyacrylamide 6% to distinguish different bands of PCR products which allowed for identifying the homozygous LCH – 01 line.

As a result, there were 3 out of 9 primers (TC4E10, TC4D02, and TC1A02) were polymorphic. In contrast, 6 primers showed the monomorphism which identical between Sen That, L18, and LCH – 01. Thus, LCH – 01 carried several drought tolerance traits of Sen That linked to 3 polymorphic primers while the results of L18 dissimilar to LCH-01 and Sen That. In this study, we used "Sen That" groundnut variety as a donor and L18 as a recipient for this cross combination. First and foremost, "Sen That" is the domestic groundnut of Nghe An, where struggles with drought annually. This domestic groundnut can adapt to the drought conditions of Nghe An which means "Sen That" contains good drought tolerance traits suitable in this region that aid the development of this groundnut variety. However, the low yield characteristic of "Sen That" is the reason for the uncommon of this variety on the field and lead to the reduction in the growing area. Thus, the use of this variety in breeding to enhance the drought tolerance ability of many commercial groundnut varieties is necessary. Even though, there is lacking application of "Sen That" variety in groundnut breeding, especially in Vietnam. And L18 import variety plays a role as recipient variety which has high productivity but drought susceptible. Under good irrigating conditions, the production of L18 is very high. The total yield of L18 variety 14.38% higher than the L14 commercial groundnut variety even the yielding factors are lower (data are not shown). Despite the high productivity of

L18, this variety requires a high amount of water for growing and developing. If the provided water did not meet the requirement, the plant can easily wilt and die. Thus, the potential this cross combination between L18 and Sen That. LCH-01 is one of many backcrossed and selfing lines generated from the L18 and Sen That crossed combination. Based on some phenotypic characteristics such as color of the leaf, the height of plant, fruit, the thickness of the groundnut shell, etc, the LCH-01 had been chosen as a potential backcrossed line which better than the other 10 backcrossed lines. As the results presented in table 2 and table 3, LCH-01 backcrossed line has drought tolerance ability better than the domestic variety. Besides, the recovered level of LCH-01 also equal to the Sen That variety but the wilted level lower than Sen That which proved for the improvement of this line. In addition, The development of LCH – 01 under drought stress conditions even higher than both parent varieties. Hence, the LCH – 01 has drought tolerance ability and development characteristics superior to the "Sen That" – the donor variety and L18 – recipient variety. With the development of advanced biotechnology, the use of SSR markers linked to drought tolerance traits is an effective approach for groundnut breeding. In this study, all 9 primers were previously published [4, 5, 6, 9]. Although, these markers did not link to specific genes regulated to the drought tolerance. Most SSR markers could link to water-use efficiency traits but had no specific genes linked to the markers. Lacking study about the physical maps of genes linked to drought tolerance traits in groundnut is a limitation in identifying the accurate genes which link to the SSR markers in the study [9].

In this study, we identified for the homozygous LCH-01 backcrossed line based on SSR markers and measured drought tolerance indexes, several issues were drawn. Firstly, the primers for SSR markers were not highly specific. The results of electrophoresis were found in the non-specific bands. The gel results must, therefore, be based on the expected product size to find the amplified sequence. Secondly, the separation of the primers' bands was not clear which electrophoresis might require in a longer period. Last but not least, the number of SSR markers used in this study just 9 primers, and 3 of them were showed polymorphisms. There might be more traits related to the drought tolerance that could occur in both Sen That, LCH – 01, and showed the polymorphism which ensured the LCH – 01 backcrossed line that carried the drought tolerance traits of Sen That.

Conclusions

In summary, we have successfully generated the elite line LCH – 01 from the BC2F3 of "Sen that" X "L18" crossing combination. By molecular applying, 3 markers were polymorphism and involved in the drought tolerance ability of this line. This line shows high drought tolerance, high yield and possibly releases to the drought areas in the coming time.

Acknowledgment. The research was funded by the Ministry of Science and Technology (MOST) under the Project Grant numbers: ĐTĐL.CN-06/16.

References

[1] P.S. Birthal, P.P. Rao, S.N. Nigam, M.C.S. Bantilan, Bhagavatula, S. Groundnut and soybean economies of Asia: facts, trends and outlook. Technical Report (2010). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.
[2] D.M. Le, Agricultural restructuring towards raising added values and sustainable development in Hanoi city, Vietnam, *IOP Conf. Ser.: Earth Environ. Sci.* (2019), 338 012023. https://doi.org/10.1088/1755-1315/338/1/012023
[3] D.T.K. Cuc, N.T.T. Loan, N.D. Cuong, N.T.T. Ngoan, N.T. Loan, D.T.C. Anh, P.T. Phuong, P.V. Linh, P.D. Trinh, N.Q. Huy, K.H. Trung, T.D. Khanh. Improving late leaf spot resistance of peanut by molecular breeding, *Adv. Stud. Biol*., **13** (2021), 11-19. https://doi.org/10.12988/asb.2021.91255
[4] M.C. Moretzsohn, L. Leoi, K. Proite, *et al.*, A microsatellite-based, gene-rich linkage map for the AA genome of Arachis (Fabaceae), *Theor. Appl. Genet.* **111** (2005), 1060–1071. https://doi.org/10.1007/s00122-005-0028-x
[5] M.E. Ferguson, M.F. Burow, S.R. Schulze, P.J. Bramel, A.H. Paterson, S. Kresovich, S. Mitchell, Microsatellite identification and characterization in peanut (Arachis hypogea L.), *Theor. Appl. Genet.*, **108** (2004), 1064-1070. https://doi.org/10.1007/s00122-003-1535-2
[6] G. He, R. Meng, M. Newman, G. Gao, R.N. Pittman, C.S. Prakash, Microsatellite as DNA Marker in cultivated Peanut (*Arachis hypogea* L.), *BMC Plant Biol.*, **3** (2003), 3. https://doi.org/10.1186/1471-2229-3-3
[7] P. Ratnakumar, V. Vadez, Groundnut (*Arachis hypogaea*) genotypes tolerant to intermittent drought maintain a high harvest index and have small leaf canopy under stress, *Func. Plant Biol.*, **38** (2011), 1016–1023. https://doi.org/10.1071/fp11145
[8] N. Thangthong, S. Jogloy, N. Jongrungklang *et al.*, Root distribution patterns of peanut genotypes with different drought resistance levels under early-season drought stress, *J. Agro. Crop. Sci.*, (2017) 1–12. https://doi.org/10.1111/jac.12249.
[9] C. Nandini, D. Savithramma, P. Doddaraju, P. Kumar, Pavan, Polymorphic SSR marker identification for water use efficiency in groundnut (*Arachis hypogea* L.) parental lines, *Legume Res - Inte Journal*, (2018). 10.18805/LR-3980

Received: January 29, 2021; Published: February 26, 2021