C. R. Biologies xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Molecular biology and genetics/Biologie et génétique moléculaires

ISSR marker-assisted genetic diversity analysis of *Dioscorea hispida* and selection of the best variety for sustainable production

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ARTICLE INFO

Article history:
Received 22 June 2017
Accepted after revision 17 August 2017
Available online xxx

Keywords:
Genetic diversity
Dioscorea hispida
ISSR markers
Breeding
Sustainable production

ABSTRACT

Plant breeding is a way of selection of a particular individual for the production of the progeny by separating or combining desired characteristics. The objective of this study was to justify different characteristics of *Dioscorea hispida* (Ubi gadong) varieties using molecular techniques to select the best variety for sustainable production at the farmer's level. A total of 160 germplasms of Ubi gadong were collected from different locations at the Terengganu and Kelantan states of Malaysia. Forty eight (48) out of 160 germplasms were selected as "primary" selection based on yield and other qualitative characters. Selected collections were then grown and maintained for ISSR marker-assisted genetic diversity analysis. Overall plant growth and yield of tubers were also determined. A total of 12 ISSR markers were tested to justify the characteristics of Ubi gadong varieties among which three markers showed polymorphic bands and on average 57.3% polymorphism were observed representing the highest variation among germplasms. The ISSR marker based on UPGMA cluster analysis grouped all 48 *D. hispida* into 10 vital groups that proved a vast genetic variation among germplasm collections. Therefore, hybridization should be made between two distant populations. The *D. hispida* is already proved as the highest starch content tuber crops and very rich in vitamins with both micro and macro minerals. Considering all these criteria and results from marker-assisted diversity analysis, accessions that are far apart based on their genetic coefficient (like DH27 and DH71; DH30 and DH70; DH43 and DH62; DH45 and DH61; DH77 and DH61; DH78 and DH57) could be selected as parents for further breeding programs. This will bring about greater diversity, which will lead to high productive index in terms of increase in yield and overall quality and for the ultimate target of sustainable Ubi gadong production.

1. Introduction

Plant breeding is defined as the selection of a particular individual for the production of progeny by separating or combining the characteristics, morphology, physiology and resistance to fungal diseases and insects. Because Ubi gadong is a perennial species and the breeding period of this species is long, Ubi gadong is not generally grown by farmers; instead it grows in a wild state in the forests and hilly lands. But it can also be grown through vegetative methods of cultivation using corms in a suitable growing

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http://dx.doi.org/10.1016/j.crvi.2017.08.003
1631-0691/

area. With the process for conventional planting, bulbs will grow and can be collected as they are, growing at wild state in the woods and abandoned areas. The period of breed selection with the desired characteristics can be shortened with the use of molecular marker techniques such as Random Amplified Polymorphic DNA (RAPD), *Inter* Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR). Molecular marker technology has been used to study the pattern of genetic inheritance in the genus *Dioscorea*, among others, *D. nebula* and *D. dumetorum* using Amplified Length Polymorphism (AFLP) [1]. In addition, this technique is useful for determining the status of a species or between species of plants. Molecular marker techniques are pleasing, fast and efficient because genetic information is preserved from any influence of the environment as compared with the morphological information. However, the combination of these two molecular and morphological techniques is a better and more effective strategy for breeding *D. hispida*. *Dioscorea* species is a rich source of starch with high caloric value, containing huge vitamins, minerals, and other pharmaceutical properties [2]. From the ancient time, it is being eaten by local people of different countries including Malaysia as an easy and cheapest source of food and different parts of this plant as a remedy for various health disorders including stomach ache, constipation, indigestion, abdominal pain, dysentery, cough, cold, asthma, tuberculosis, skin wounds, boils, sunburn, reducing body heat, and many more [3–5]. But this important crop is still underutilized and there is no improved variety for commercial cultivation. So, the selection of new varieties of *D. hispida* characterized by high yield and rapid growth is very essential. Considering the importance of the above, this study was conducted to choose a potential breed of *D. hispida* using conventional methods and ISSR markers assessing genetic diversity and patterns of inheritance among the collected germplasm collections. The final goal is the propagation of the selected breed and the supply of seedlings to the farmers for improved cultivation and sustainable production of *Dioscorea hispida*.

2. Materials and methods

2.1. Germplasm collection and evaluation

A total of 160 Ubi gadong accessions were collected from two states of Malaysia: Terengganu and Kelantan. Samples were collected from five different districts and 11 villages and weighing about 423.5 kilograms of corms (yellow and white, two different varieties of same species) obtained during sampling. Then, selections were done on all samples based on the desired characteristics of a high tree with high yield containing lot of corms. Then, as many as 48 samples from 160 Ubi gadong germplasms were selected as "primary" samples, which were then grown and maintained in Germplasm plot, Research Farm in Gong Badak campus, Universiti Sultan Zainal Abidin. Complete herbarium specimens with male and female flowers were also stored in the herbarium for future identification in Universiti Sultan Zainal Abidin, Kuala Terengganu and Universiti Kebangsaan Malaysia, Bangi. Brief collection details of Ubi gadong accessions are displayed in Table 1.

Table 1
Brief collection details of the collected Ubi gadong samples.

No.	Accession	District	Villages
1	DH 0027	Marang	KG Sungai Serai
2	DH 0028	Marang	KG Sungai Serai
3	DH 0029	Marang	Bukit Belacan, KG Sungai Serai
4	DH 0030	Marang	Bukit Belacan, KG Sungai Serai
5	DH 0031	Marang	Bukit Belacan, KG Sungai Serai
6	DH 0032	Marang	Bukit Belacan, KG Sungai Serai
7	DH 0033	Marang	Bukit Belacan, KG Sungai Serai
8	DH 0034	Marang	Bukit Belacan, KG Sungai Serai
9	DH 0035	Marang	Bukit Belacan, KG Sungai Serai
10	DH 0036	Marang	Bukit Belacan, KG Sungai Serai
11	DH 0037	Marang	Bukit Belacan, KG Sungai Serai
12	DH 0038	Marang	Bukit Belacan, KG Sungai Serai
13	DH 0039	Marang	Bukit Belacan, KG Sungai Serai
14	DH 0040	Marang	Bukit Belacan, KG Sungai Serai
15	DH 0041	Marang	Bukit Belacan, KG Sungai Serai
16	DH 0042	Marang	Bukit Belacan, KG Sungai Serai
17	DH 0043	Marang	Bukit Belacan, KG Sungai Serai
18	DH 0044	Marang	Bukit Belacan, KG Sungai Serai
19	DH 0045	Marang	Bukit Belacan, KG Sungai Serai
20	DH 0046	Marang	Bukit Belacan, KG Sungai Serai
21	DH 0047	Marang	Bukit Toktong, KG Sungai Serai
22	DH 0048	Marang	Bukit Toktong, KG Sungai Serai
23	DH 0049	Marang	Bukit Toktong, KG Sungai Serai
24	DH 0050	Marang	Bukit Toktong, KG Sungai Serai
25	DH 0051	Marang	Bukit Toktong, KG Sungai Serai
26	DH 0052	Marang	Bukit Toktong, KG Sungai Serai
27	DH 0053	Marang	Bukit Toktong, KG Sungai Serai
28	DH 0054	Marang	Bukit Toktong, KG Sungai Serai
29	DH 0055	Marang	Bukit Toktong, KG Sungai Serai
30	DH 0056	Marang	Bukit Toktong, KG Sungai Serai
31	DH 0057	Marang	Bukit Toktong, KG Sungai Serai
32	DH 0058	Marang	Bukit Toktong, KG Sungai Serai
33	DH 0059	Marang	Bukit Toktong, KG Sungai Serai
34	DH 0060	Marang	Bukit Toktong, KG Sungai Serai
35	DH 0061	Marang	Bukit Toktong, KG Sungai Serai
36	DH 0062	Marang	Bukit Toktong, KG Sungai Serai
37	DH 0063	Marang	Bukit Toktong, KG Sungai Serai
38	DH 0064	Marang	Bukit Toktong, KG Sungai Serai
39	DH 0070	Hulu TRG	KG Pangkalan AJAL
40	DH 0071	Hulu TRG	KG Pangkalan AJAL
41	DH 0072	Hulu TRG	KG Pangkalan AJAL
42	DH 0073	Hulu TRG	KG Pangkalan AJAL
43	DH 0074	Hulu TRG	KG Pangkalan AJAL
44	DH 0075	Hulu TRG	KG Pangkalan AJAL
45	DH 0077	Hulu TRG	KG Pangkalan AJAL
46	DH 0078	Hulu TRG	KG Pangkalan AJAL
47	DH 0079	Hulu TRG	KG Pangkalan AJAL
48	DH 0080	Besut	KG Air Terjun

2.2. Propagation, growth and data collection

As Ubi gadong usually produce corms instead of normal seeds like other crops, vegetative propagation was followed using corms/bulbs. Sampled bulbs were planted in polybags (15×18 inches), weighing 200–300 g each using a prepared soil (soil:sand:manure = 3:2:1) with five replications. Growing areas were covered with 50% shade and light using black shade cloths until proper maturity of the Ubi gadong plants. Another method of preparation of propagating materials is; to cut corm with respect to some parts of the form and number of buds that form on the surface of the skin of the corm. Transplanted bulbs were tagged and labeled properly. All other agronomic practices (weeding, irrigation, fertilizer application etc.) were done following standard methods. Data on tree height and corm weight were observed every

Please cite this article in press as: N.F.H. Nudin, et al., ISSR marker-assisted genetic diversity analysis of *Dioscorea hispida* and selection of the best variety for sustainable production, C. R. Biologies (2017), http://dx.doi.org/10.1016/j.crvi.2017.08.003