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Improving the Dry Matter Content of Pumpkin Lines by Conventional Breeding

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Abstract Pumpkin (Cucurbita spp.) is one of the most important vegetable crops due to its rich in healthy nutrition. This study aimed to develop F1, F2 and F3 by crossed between the low and high dry matter contents of parental plants. A total of 200 individuals of F2 attained from F1, then made selfing to develop F3 populations. Generally, the dry matter contents of F3 lines were ranged from 3.7% to 11.3% and the average value was 7.3%. Eightlines of F3 showed high dry matter potential contents and were selected including B20 (11.3%); B21 and B101 (10.5%); B1 (10.3%); B17 (10.2%); B82 (10.0%); B46 and B106 (9.8%), respectively. The results of this study have provided useful information and initial materials to further identify the traits of pumpkin quality to improve the high dry matter contents.

Keywords Pumpkin, breeding, population, high dry matter content

Introduction

Pumpkin (*Cucurbita* spp.) is considered a functional vegetable in the *Cucurbitaceae* family with over 130 genera and 800 species [1]. Due to its wide range of adaptability to climate conditions, pumpkin is growing in many areas globally. According to the report of Atlasbig [2], the total worldwide pumpkin is yearly produced approximately 26.5 million tones, in which, China and India are the largest pumpkin producer countries with an estimation of 7.8 and 5.07 million tones.

In Vietnam, pumpkin is considered a secondary crop, hence, there are no specific statistics available on both growing areas and total yield. However, according to some different recent studies, pumpkin cultivation in this country is still small-scale, unfocused areas and spontaneously developed by farmers. In fact, pumpkin or squash has been cultivated for a long time in Vietnam and is grown in many areas across this country included in the outback and moutainous areas. In Vietnam, the approaches and strategies of vegetable breeding have started in the last decades and focused on improving the quality of vegetables. Specifically, from 1968 to 1985, most works were concentrated on material collection, tested and selected the traits of interests such as high yield, vigor growth of the native and traditional varieties. From 1986 to 1995, significant enhancements were made with the attempts to select and generate the pure vegetable varieties via conventional breeding. From 1996 to 2000, the first F1 hybrid of vegetables was generated [3]. Presently, the trend of main studies and goalsare approached to increase the high quality and tolerant ability of vegetable plants against the abiotic and biotic stresses.

Recently, pumpkin is considered as one of the important vegetables for daily meals and has brought high economic value to farmers [4-5]. Pumpkin is a crop with high production efficiency because its parts such as stems, leaves, flowers and seeds can be used as food and raw materials for the confectionery, oil pressing industries, etc., [5]. Numerous reports showed that pumpkin has rich in vitamin and secondary metabolites, and potential medical properties which are promoted human health, for example, antioxidant, antimicrobial, antifungal, antimalarial, antihelmintic, antifatigue, antidiabetic, anticancer, antiviral, antibladder stone, enzymatic inhibitor and antiulcer [6]. In Vietnam, a recent study by Hue et al [7] reported that Vietnamese pumpkin has potential vitamin A, C, proteins, fat, sugar, high content of dry matter, etc., and provides high energy with 85170kJ/100g and young leaves, stems and growing tips of pumpkin are traditionally eaten as the daily vegetables [7]. With rich in pumpkin germplasms and high diversity in this country, pumpkin is grown a long time ago, however, very few studies on pumpkin are available, especially evaluating the dry matter content and the quality of pumpkin. Therefore, the objective of this study was to evaluate the high dry matter contents from F2 and F3 populations developed by crossing between the low and high dry matter contents of parental pumpkin varieties.

Materials and Methods

Material collection

In this study, 12 pumpkin varieties were kindly provided by the Plant Resource Center. The variety name, collected areas, and their dry matter contents were described in Table 1.

Table 1: List of pumpkin varieties and their dry matter contents were used in this study

No	Code	Variety name	Collection province	Dry matter content (%)*
1	SĐK 3630	Bido gao	Thai Nguyen	13.3
2	SĐK 3862	Bi do	Quang Tri	14.0
3	SĐK 5363	Nung lang cao	Bac Giang	12.0
4	SĐK 6723	Bi u	Ha Giang	12.3
5	SĐK 6916	Bido qua dai	Thanh Hoa	13.5
6	SĐK 8382	Ma u	Son La	11.5
7	SĐK 8387	Nhum xi	Son La	19.0
8	SĐK 6555	Bi do	Ha Giang	4.0
9	SĐK 7529	Bio	Bac Giang	3.8
10	SĐK7546	Xeng to	Hoa Binh	4.0
11	SĐK 8571	Bi do	Kon Tum	4.0
12	SĐK 15082	Mac uc	Thanh Hoa	4.3

*: *Dry matter contents of those varieties were provided by the Plant Resource Center [9].*

Based on their dry matter contents, the crossed combinations between the low and high dry matter contents of those varieties were selected. The breeding schedule was presented in Table 2

Table 2: Pumpkin hybrids to create hybrid populations in the direction of dry matter content

Female variety	Male variety				
	SĐK6555	**SĐK7529**	**SĐK7546**	**SĐK8571**	**SĐK15082**
SĐK3630	THL1*	THL8	THL15	THL22	THL29
SĐK3862	THL2	THL9	THL16	THL23	THL30
SĐK5363	THL3	THL10	THL17	THL24	THL31
SĐK6723	THL4	THL11	THL18	THL25	THL32
SĐK6916	THL5	THL12	THL19	THL26	THL33
SĐK8382	THL6	THL13	THL20	THL27	THL34
SĐK8387	THL7	THL14	THL21	THL28	THL35

**: THL implies the breeding combination*

Methods

Experiment Design

The pumpkin seeds were used as the parental varieties were sown in the plastic trays with 5 individuals for each variety. When the seedlings reached 2-3 true leaves, each variety wassown in the separate beds, planted in 2 rows with the distance 2m of each variety. The experimental plot area was prepared by 30 m^2 (10 m x 3 m). The planting bed was ranged approximately 3 m in width, the trench was 1 m wide, and the bed was 0.3 m in height. Ten plants per plot were made. Planting techniques and experimental performance were done following the method of Plant Resource Center [9]. The amount of fertilizer for 1 ha was: 25 tons of manure + 250 kg of urea + 450 kg of superphosphate + 300 kg of potassium. Bioassays and field experiments were performed in the laboratory and the experimental station of Plant Resource Center and Vietnam Academy of Agricultural Sciences (VAAS) during 2018 and 2019.

Hand pollination and evaluation of dry matter contents of crossing population

During the flowering period, the well-grown plants with few or without pests and disease infestation were selected. Breeding method was done following the conventional method of the Plant Resource Center [9] as shown in Figure 1. Specifically, the selected flowers to cross must be developed normally. The female flowers were hand-pollinated by male parents. After that, the paper bags were used to cover the pollinated flowers to avoid outcrossing by wind or insects.

A **B** **C**

Figure 1: Pumpkin flowers used for breeding; A: Male flower, B: female flower; C: Hand pollination of pumpkin

After successful pollination, the pumpkin fruits were considered as F1 offsprings. The seeds of F1 were planted and selfed pollinated to develop F2 and F3 populations. The dry matter content of the crossed lines and F3 populations, their fruits were dried at 105^{o}C according to the method of National Standard - TCVN 10696:2015.2.2.3 [10].

Statistical Analysis

The raw data were analyzed by using the Excel ver 2016.

Results and Discussion

Evaluation of time to attain F1 fruits among the crossed combinations

In this study, we conducted crossing combinations between the parental varieties with high dry matter and low dry matter contents. The results of F1 crossed combination were presented in Table 3.

Table 3: Evaluation of F1 fruits among the crossed combinations

No	Code of crossed combination	Total flower	Number of fruits that develop normally after days-calculation							
			5 days		10 days		15 days		30 days	
			Number of fruit	Rate (%)	Number of fruit	Rate (%)	Number of fruit	Rate (%)	Number of fruit	Rate (%)
1	THL1	10	10	100	7	70.0	2	20.0	1	10.0
2	THL2	10	7	70.0	4	40.0	1	10.0	0	0
3	THL3	20	15	75.0	10	50.0	5	25.0	1	5.0
4	THL4	20	17	85.0	9	45.0	6	30.0	3	15.0
5	THL5	10	10	100	7	70.0	2	20.0	1	10.0
6	THL6	10	9	90.0	5	50.0	3	30.0	2	20.0
7	THL7	4	4	100	1	25.0	0	0	0	0
8	THL8	5	3	60.0	3	60.0	0	0	0	0
9	THL9	10	7	70.0	5	50.0	1	10.0	1	10.0
10	THL10	9	9	100	4	44.4	1	11.1	2	22.2
11	THL11	7	3	42.9	3	42.9	1	14.3	1	14.3
12	THL12	3	3	100	1	33.3	0	0	0	0
13	THL13	7	7	100	4	57.1	2	28.6	1	14.3
14	THL14	8	8	100	6	75.0	5	62.5	2	25.0
15	THL15	2	2	100	1	50.0	0	0	0	0
16	THL16	3	3	100	2	66.7	0	0	0	0
17	THL17	5	4	80.0	3	60.0	1	20.0	1	20.0
18	THL18	6	4	66.7	3	50.0	3	50.0	1	16.7
19	THL19	11	8	72.7	5	45.5	2	18.2	2	18.2
20	THL20	9	9	100	5	55.6	3	33.3	0	0
21	THL21	6	5	83.3	4	66.7	3	50.0	0	0
22	THL22	8	8	100	7	87.5	7	87.5	5	62.5
23	THL23	9	6	66.7	3	33.3	2	22.2	2	22.2
24	THL24	11	9	81.8	5	45.5	4	36.4	2	18.2
25	THL25	16	11	68.8	5	31.3	2	12.5	1	6.3
26	THL26	8	8	100	2	25.0	1	12.5	1	12.5
27	THL27	10	7	70.0	4	40.0	2	20.0	1	10.0
28	THL28	4	3	75.0	2	50.0	1	25.0	0	0
29	THL29	7	7	100	3	42.9	1	14.3	1	14.3
30	THL30	9	9	100	3	33.3	2	22.2	2	22.2
31	THL31	3	2	66.7	0	0	0	0	0	0
32	THL32	11	11	100	5	45.5	3	27.3	2	18.2
33	THL33	8	5	62.5	4	50.0	2	25.0	2	25.0
34	THL34	2	2	100	0	0	0	0	0	0
35	THL35	9	9	100	6	66.7	3	33.3	1	11.1

The results showed that there was a significant difference among the number of crossed flowers due to their characteristics of pumpkin flowers. For instance, the male flowers were approximately 20 times over the female flowers. On the other hand, male and female flowers bloom unevenly, the male flowers could bloom two to three days before female flowers, and pollen grains can only be pollinated for a few hours, so the matching rate in each combination is quite different and high variation. To overcome this situation, when making a cross, it is advisable to sow the parent lines into different days and should be sown from 5-7 days apart. As observed from the formation and development of F1 fruit, we found that: at 5 days after pollination, the percentage of flower and fruit development was rather high ranged from 42.9 -100%. The rate of normal and developed fruits gradually decreased over the days of monitoring and remarkably decreased after 10 days after pollination, followed by 15 days and reached stability after 30 days of pollination.

As the result attained, at 10 days after pollination, only 1 combination named THL31 of (SDK5363 x SDK15082) was 2.9% and all fruits gradually appeared yellowed and withered. Contradically, at 15 days after pollination, the 7 crossed combinations were 20%, however, the phenomenon of all crossed fruits was gradually changed in yellow and wilting. It noted that at 30 days after pollination, there were 11 combinations were stabilized and developed (Figure 2). It can be explained that due to incompatibility in the crossing process or because of some external factors such as temperature, humidity and light, etc. Therefore, in order to increase the number of F1, it is necessary to increase the number of flowers/combinations by arranging cross-breeding experiments with different parental sowing times to prolong the overlapping flowering time of the lines.

Figure 2: F1 fruit and seeds of pumpkin

Number of filled fruits and seeds of F1 fruits

The results showed that there were 24 out of 35 crossed combinations, accounted for 69%, which were successfully crossed to obtain F1 seeds; the total number of fruits obtained from each crossed combination was from 1 to 5 fruits. The number of F1 seeds obtained from each crossed combination was ranged from 28 to 220 seeds; in which, the most combination P22 (3630 x 8571) obtained 220 seeds. There were 13 other crossed combinations, accounted for 37%, only 1 fruit was fewer seeds ranged from 28 - 51 seeds/fruit. There were 9 hybrid combinations, accounting for 26%, and obtained 2 fruits with the number of F1 hybrid seeds from 67 - 83 seeds/fruit, and the other 3 fruits obtained from one crossed combination were 87 seeds per fruits, respectively (Table 4).

Table 4: Number of fruits and their seeds of F1 obtained from the crossed combinations

No	Code of cross combination	Number of fruit	Number of F1 seeds	No	Code of cross combination	Number of fruit	Number of F1 seeds
1	THL1	1	28	19	THL19	2	83
2	THL2	0	0	20	THL20	0	0
3	THL3	1	38	21	THL21	0	0
4	THL4	3	87	22	THL22	5	220
5	THL5	1	34	23	THL23	2	87
6	THL6	2	76	24	THL24	2	71
7	THL7	0	0	25	THL25	1	42
8	THL8	0	0	26	THL26	1	45
9	THL9	1	41	27	THL27	1	41

No	Code of cross combination	Number of fruit	Number of F1 seeds	No	Code of cross combination	Number of fruit	Number of F1 seeds
10	THL10	2	74	28	THL28	0	0
11	THL11	1	28	29	THL29	1	51
12	THL12	0	0	30	THL30	2	67
13	THL13	1	29	31	THL31	0	0
14	THL14	2	67	32	THL32	2	75
15	THL15	0	0	33	THL33	2	71
16	THL16	0	0	34	THL34	0	0
17	THL17	1	40	35	THL35	1	48
18	THL18	1	43				

Among the 35 crossed combinations, there was a THL22 which was crossed between the varieties ĐK3630 and ĐK8571 and gained a total of 220 F1 seeds. These seeds were then selfed and developed F2 and F3 populations. In summary, we successfully developed F2 generation and attained a total of 200 F2 seeds. Those seeds were continuously developed F3 generations and selected the potential F3 lines.

Evaluation of dry matter content of F3 lines

In pumpkin, the quality and nutrient compositions are frequently determined by its dry matter content, Brix content, vitamin C and beta carotene contents. However, the quality of pumpkin is largely depended on the variety, harvest time and storage [6]. In this study, the dry matter content was calculated by the weight of fresh fruit pulp and edible parts in ripe F3 lines. We have evaluated the dry matter contents of 120 F3 lines as presented in Table 5 and Figure 3. Generally, the dry matter contents of 120 F3 lines were ranged from 3.7 to 11.3%. Statistical results showed that approximately 73% of F3 lines has had dry matter content ≥ 6% and 27% of F3 lines has had dry matter content < 6%, respectively. Interestingly, we have found 8 lines of F3 with high dry matter contents, including B20 (11.3%); B21 and B101 (10.5%); B1 (10.3%); B17 (10.2%); B82 (10.0%); B46 and B106 (9.8%), respectively.

Table 5: Evaluation of dry matter contents of 120 lines of F3

No	F_3 line	Dry matter content (%)	No	F_3 line	Dry matter content (%)
1	B1	10.3	61	B84	4.8
2	B3	8.4	62	B85	4.4
3	B4	9.3	63	B86	8.3
4	B5	9.5	64	B87	5.6
5	B8	6.5	65	B89	5.6
6	B9	7.2	66	B90	7.4
7	B10	7.2	67	B91	3.8
8	B11	8.8	68	B92	6.5
9	B12	4.8	69	B95	6.9
10	B13	3.9	70	B96	9.5
11	B14	5.8	71	B97	5.3
12	B15	9.5	72	B98	9.4
13	B16	9.7	73	B99	7.3
14	B17	10.2	74	B100	8.1
15	B18	8.3	75	B101	10.5
16	B19	7.9	76	B103	4.2
17	B20	11.3	77	B104	9.5
18	B21	10.5	78	B106	9.8
19	B22	7.1	79	B107	8.3
20	B23	7.7	80	B109	7.7
21	B24	4.2	81	B110	5.7
22	B25	6.5	82	B113	7.1

No	F_3 line	Dry matter content (%)	No	F_3 line	Dry matter content (%)
23	B26	8.5	83	B114	8.2
24	B28	4	84	B115	7.3
25	B29	9.5	85	C116	5.5
26	B30	7.2	86	B117	5.4
27	B31	7.5	87	B120	7.6
28	B33	6.5	88	B121	7.6
29	B34	9.5	89	B122	7.9
30	B35	7.6	90	B123	8.1
31	B36	7.5	91	B124	8.2
32	B39	6.7	92	B125	5.5
33	B40	7.9	93	B127	9
34	B42	4.2	94	B128	5.9
35	B45	8	95	B130	7.6
36	B46	9.8	96	B131	4.3
37	B49	5.7	97	B133	6.8
38	B50	6.9	98	B134	7.5
39	B51	7.9	99	B136	8.1
40	B53	6.4	100	B142	4.4
41	B54	8.3	101	B147	4.2
42	B55	8.9	102	B149	7.3
43	B59	3.7	103	B150	6.7
44	B60	4.5	104	B151	6.5
45	B61	9.1	105	B158	7.2
46	B62	4.2	106	B160	8.5
47	B63	8.7	107	B161	7.7
48	B64	7.9	108	B162	9.1
49	B65	4.1	109	B165	9
50	B67	5.2	110	B170	7.7
51	B68	9.4	111	B176	6.9
52	B69	7.6	112	B178	4.5
53	B70	5	113	B179	9.3
54	B72	8.7	114	B180	5.8
55	B74	7.7	115	B186	9.6
56	B75	5.3	116	B188	8.5
57	B80	4.9	117	B194	7.5
58	B81	8.9	118	B195	7.8
59	B82	10	119	B197	8.1
60	B83	5.1	120	B199	8.3
Female parent	SĐK3630	13.3			
Male parent	SĐK8571	4			
Min					3.7
Max					11.3
Average					7.3

The promising lines can be used to develop the high quality of pumpkins and further identified the responsible genes/QTL to control the trait of the high dry matter content of pumpkins. In the fact that pumpkin fruits are generally high in moisture contents approximately ranged from 83 to 88%. In which the crude protein content has fluctuated from 14 to 17% on a dry matter basis and the *in vitro* digestibility, where the dry matter content is one of the key components for chemical compositions as well as for quality of pumpkin products [11-12].

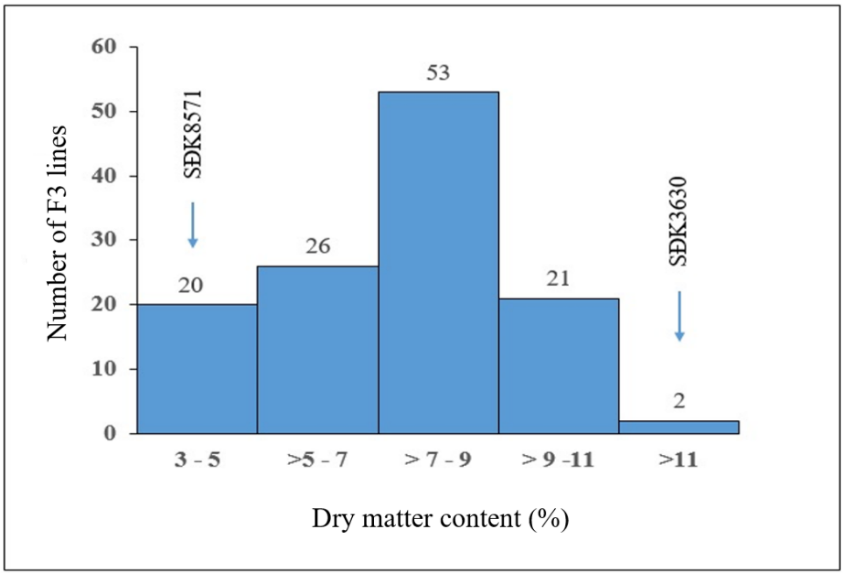

Figure 3: Frequency distribution of pumpkin F3 lines in terms of dry matter content

Conclusions

In summary, we have successfully developed F1, F2 and F3 by crossedamong the low and high dry matter contents of parental plants. As a result attained, 200 individuals of F2 were developed. The dry matter content of 120 lines of F3 pumpkin derived from a crossed combination between ĐK3630 and ĐK8571 was ranged from 3.7% to 11.3%. Eight potential lines of F3 with high dry matter contents were found including B20 (11.3%); B21 and B101 (10.5%); B1 (10.3%); B17 (10.2%); B82 (10.0%); B46 and B106 (9.8%). Our results may provide useful information on pumpkin breeding to improve the dry matter content and further identify the responsible genes/QTLs involve in the quality traits.

References

[1]. Gutierrez, R.M.P. (2016). Review of *Cucurbita pepo* (pumpkin) its phytochemistry and pharmacology. *Med.Chem*, 6(1): 12–21.

[2]. Atlasbig (2020). Worldwide pumpkin production: https://www.atlasbig.com/en-gb/countries-by-pumpkin-production (latest accession, May 17, 2021).

[3]. Ha, N.T., Van, D.T., Thanh, N.D. (2010). Vegetable course. *Agriculture Publishing, 2010* (*in Vietnamese*).

[4]. Phong, L.T, Tuong, L.K., Dao, D.V (2011). Pumpkin production, potential and challenges. *Viet J. Agric Sci. Technol*, 2: 46-50. (*in Vietnamese with English abstract*).

[5]. Phuc, N.T.T., Chi, V.L., Thuy, N.T.K., Nghia, L.T. (2017). Initial assessment of some pumpkin varieties at the National Gene Bank. *Viet J. Agric Sci. Technol*, 8: 31-36. (*in Vietnamese with English abstract*).

[6]. Sharma, P., Kaur, S., Kehinde, B.A., Chhikara, N., Panghal, A., Kaur, H. (2019). Pharmacological and biochemical uses of extracts of pumpkin and its relatives and application in the food industry: a review. *Inter J. Vegetable Sci*, DOI: 10.1080/19315260.2019.1606130.

[7]. Hue, H.T., An, N.N., Phuc, N.T.T, Huong, T.T.H, Nghia, L.T. (2020. Evaluation of morphological and agronomic characteristics of pumpkin genetic resources collected in the northern mountainous areas of Vietnam.Viet J. Agric Sci. Technol, 12: 25-32. (*in Vietnamese with English abstract*).

[8]. Plant Resource Center (2021). Annual report in vegetables

[9]. Plant Resource Center (2015). Planting techniques and experimental methods.

[10]. Fruit and vegetable juices-Determination of total dry matter-Gravimetric method with loss of mass on drying. National Standard- TCVN 10696:2015.2.2.3.

[11]. Palaitytė, G., Kubilienė, E., Marcinkonis, (2017). Positive effect of compost application on oil pumpkins growth and zinc enrichment. *Acta Biologica Universiitatis Daugavpiliensis*, 17(1).
[12]. Jenkins, K.H. (2021). Salvaging leftover pumpkins for beed cattle: https://newsroom.unl.edu/announce/beef/4452/25555#:~:text=Pumpkins%20are%20high%20in%20moisture,acre%20for%20cattle%20to%20consume.&text=The%20crude%20protein%20content%20tends,)%20is%2060%2D70%25 (latest accession, May 17, 2021).