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Identification of SSR Markers for Genetic Purity Testing in Waxy Corn F1 Hybrid Seeds

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ABSTRACT

Genetic purity is a must for the commercialization of any hybrid seeds. In order to identify a pure hybrid, morphological evaluation of seeds from grow-out-test is mandatory in Malaysia, however, the procedure is time- and money-consuming. This study aimed to identify suitable SSR markers for assessing the genetic purity of F1 hybrid seeds in waxy corn (*Zea mays* L. var. *ceratina*). Genomic DNA of ten waxy corn hybrids along with their parental lines and three commercial hybrids was extracted from seeds using DNeasy Plant Mini Kit. Ten SSR primers (umc2366, bnlg2181, bnlg2162, umc1005, phi011, umc1196, umc2077, phi112, umc1153 and bnlg381) were screened by PCR amplification, and only one primer (bnlg381) produced complementary banding pattern of both parental lines, which made a way to identify the hybrid. The bnlg381 amplified DNA band at 300bp in the female parent BELLA 1-8 and at 200bp in the male parent BELLA 1-7. The hybrid BELLA 1-8 x BELLA 1-7 has both DNA bands from its parents at 300bp and 200bp, confirming the genetic purity of this hybrid seed. The hybrid seed industry will benefit greatly from the SSR marker identified in this study, which will enable a cheaper and efficient selection of parental lines and evaluation of hybrid seeds in waxy corn breeding programs.

Keywords: Hybrid, Waxy corn, SSR markers, Genetic purity

INTRODUCTION

Waxy corn (*Zea mays* L. var. *ceratina*) often referred to as sticky corn, is economically, nutritionally, and processively valuable crop. The sticky quality of corn kernels is attributed to the nearly 100% amylopectin-containing starch in the endosperm of waxy corn (Dong et al., 2019; Kim et al., 2021). Waxy corn is rich in anthocyanins in the kernel and cob, which can be used as a viable source of antioxidants (Harakotr et al., 2016). It is consumed mainly in Asia as a foodstuff, but is also used in the textile and paper industries. Waxy corn was discovered in China in 1908, and subsequently it was also found in other Asian countries (Luo et al., 2020). Hybrid corn with high yields, excellent quality, and unique traits dominates global corn production. A single hybrid corn is produced by combining two pure lines with excellent combining ability (Hung et al., 2012).

Maintenance of genetic purity in the parental inbred lines and genetic purity testing in the resultant F1 hybrids are important aspect in corn hybrid breeding (Fernandez et al., 2023). The assessment becomes crucial because of the strict intellectual property regulations governing plant breeding, variety registration and seed certification (Sendekie, 2020). To determine the purity of F1 hybrids, the grow-out test is traditionally performed in the field. However, this test is very time and resource consuming (Elci & Hancer, 2015) as well as environmental dependence (Pallavi et al., 2011). DNA-based molecular markers are more dependable and take less time to develop; they are also not stage or tissue specific, and they are unaffected by the environment (Sudharani et al., 2012). Therefore, molecular approach is more reliable. Simple sequence repeats (SSR), commonly known as microsatellites, are a type of genetic marker that has been found to be numerous and widely distributed across the plant genome. They are co-dominant markers, able to detect large levels of allelic diversity, and are efficiently tested by polymerase chain reaction (PCR). SSR markers have been employed to assess the genetic purity of F1 hybrid seeds in various crops such as Cucurbitaceous family (cucumber, musk melon and bitter gourd) (Kiruthika & Padmanabha, 2018), maize (Chaudhary et., 2018), Solanaceae family (tomato, chilli and brinjal) (Padmanabha & Kiruthika, 2018) and small wax gourd (Chen et al., 2020). In maize, SSR markers are reliable for identification of both parental alleles, confirming the purity of F1 hybrids and diversity of maize varieties (Shinde et al., 2021). Thus, the present study was conducted to identify a specific SSR marker for hybrid purity testing in waxy corn F1 populations.

MATERIALS AND METHODS

Plant materials

The study included a total of six inbreds, ten F1 hybrids, and three commercial hybrids as listed in Table 1. The corn kernels of these F1 hybrids, their parental lines, and commercial hybrids as controls were collected from Green World Genetics Sdn. Bhd. (GWG) farm, Setiu, Terengganu, Malaysia. Each individual kernels were removed from the cobs and ground into powder with sea sand prior to DNA extraction.

Table 1. List of waxy corn and grain corn F1 hybrids along with their inbred lines and commercial hybrids used in this study.

Type	Name	Variety	Kernel's colour
Inbreds	BELLA 1-7	Waxy corn	Dark purple
	BELLA 1-8	Waxy corn	Purple
	BELLA 1 -10	Waxy corn	Yellow
	BELLA 1-12	Waxy corn	Yellow
	GWT 46-1b	Maize	Orange
	GWT 46-10b	Maize	Orange
F1 Hybrids	BELLA 1-7 x BELLA 1-8	Waxy x Waxy	Dark purple
	BELLA 1-8 x BELLA 1-7	Waxy x Waxy	Purple
	BELLA 1-12 x BELLA 1-8	Waxy x Waxy	Orange
	BELLA 1-8 x BELLA 1-12	Waxy x Waxy	Purple
	BELLA 1-8 x BELLA 1-10	Waxy x Waxy	Purple
	BELLA 1-10 x BELLA 1-8	Waxy x Waxy	Yellow
	BELLA 1-12 x GWT 46-1b	Waxy x Waxy	Yellow
	GWT 46-1b x BELLA 1-12	Maize x Waxy	Yellow
	BELLA 1-12 x GWT 46-10b	Waxy x Maize	Yellow
	GWT 46-10b x BELLA 1-12	Maize x Waxy	Yellow
Commercial Hybrids	C1	Waxy corn	White + yellow + purple
	C2	Waxy corn	White + purple + yellow
	C3	Waxy corn	White + yellow + purple

DNA Extraction and SSR-PCR Amplification

Genomic DNA from the kernels was extracted using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol and then stored at -20 ℃ until use. Ten SSR primer pairs were screened to discriminate the hybrids from their parents. SSR primers were selected from the literatures (Table 2), and the selection criterion was based on high polymorphism. The PCR reaction was performed using a total volume of 25 µl containing 2.5 µl of 10X buffer, 0.5 µl of 10µM dNTPs, 0.1U of *Taq* DNA polymerase, 0.5 µl of 10 µM of each forward and reverse primer, 1 µl of DNA template (30ng/µl), 1.5 µl of 1.5 mM $MgCl_2$, and 18.4 µl of sterile distilled water. All PCR reagents were purchased from New England Bio Labs Inc (NEB Biolabs, Beverly, MA, USA). The PCR reaction was performed using 96-well Fast Thermal Cycler (Applied BiosystemTM VeritiTM) with the following PCR profile: an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, primer annealing at 58 °C for seconds, extension at 72 °C for 40 seconds, and a final extension at 72 °C for 8 minutes. The same annealing temperature was used for all the primers. This method was adapted from Devi et al. (2017) with modifications. PCR products were then visualized on a 2% (w/v) agarose gel stained with CSL Runsafe (Cleaver Scientific Ltd). The agarose gel was run with 1X TBE buffer at 100 volts for 1 hour. The gel was then viewed under a UV transilluminator (LAS 4000 Gel Imager, Fujifilm).

Table 2. List of SSR primers used in this study.

No	Primers	Sequences (5'-3')	References
1	umc2366	F: ACATCGATCCAACCGTCATAAATC	
		R: CCTTCTTCCCGTCATTCTTCTTCT	Sa et al., 2010
2	umc1005	F: TTTGATCACAGACTTATCCCTGTT	
		R: CTAATGACGAACCCCTAAAAGGT	
3	umc2077	F: AAACTCACTGAACATGATCCTGGC	Sivaranjani et al., 2014
		R: CTGGTTCGGATGCAAGTAGTCA	
4	bnlg2181	F: CCAATTCACCAATCATGCAA	
		R: TTGGGGTGAAGCAATGTGTA	
5	phi011	F: TGTTGCTCGGTCACCATACC	
		R: GCACACACACAGGACGACAGT	
6	umc1196	F: CGTGCTACTACTGCTACAAAGCGA	Zheng et al., 2013
		R: AGTCGTTCGTGTCTTCCGAAACT	
7	bnlg2162	F: GTCTGCTGCTAGTGGTGGTG	
		R: CACCGGCATTCGATATCTTT	
8	phi112	F: TGCCCTGCAGGTTCACATTGAGT	
		R: AGGAGTACGCTTGGATGCTCTTC	
9	umc1153	F: CAGCATCTATAGCTTGCTTGCATT	
		R: TGGGTTTTGTTTGTTTGTTTGTTG	
10	bnlg381	F: TCCCTCTTGAGTGTTTATCACAAA	
		R: GTTTCCATGGGCAGGTGTAT	

The band produced were observed to determine the size of allele between the F1 hybrids and its parental lines. SSR markers that generate complementary banding pattern between the hybrids and its parental lines were used as the marker to test the purity of F1 hybrid seeds (Chaudhary et. al., 2018).

Validation of selected SSR marker for hybrid seed purity testing

To validate the suitability of selected SSR marker and to ensure the genetic purity of waxy corn hybrids, sequential assessment was carried out using F1 hybrid BELLA 1-8 x BELLA 1-7. The seeds were randomly selected from the seed lot of F1 hybrid BELLA 1-8 x BELLA 1-7. The genomic DNA was isolated from

individual hybrid seeds and further subjected to SSR-PCR amplification using the hybrid specific marker. The SSR banding patterns of hybrids were then compared to their parental lines.

RESULTS AND DISCUSSION

DNA Extraction and SSR-PCR Amplification

Identification and characterization of plant varieties are important for plant breeding, variety release, and seed certification programs. Maintaining seed purity is crucial for optimal crop production. In Malaysia, the conventional grow-out test is used to assess the seeds genetic purity based on the plant phenotype. However, this method is time-consuming and costly. Moreover, the morphological markers are often influenced by the environmental conditions and the results are considered subjective (Kumar et al., 2022). To overcome these limitations, DNA markers particularly the co-dominant markers have been used to distinguish hybrids, its inbred lines and off-types (Kovincic et al., 2023; Shinde et al., 2021; Chen et al., 2020). Due to their great efficacy, reproducibility, and simplicity, microsatellite markers have emerged as the preferred molecular markers for genetic purity testing (Bhat et al., 2017). According to Chaudhary et al. (2018), PCR-based co-dominant SSRs are recommended for genotyping due to their repeatability and suitability for high-throughput screening. The present study demonstrated the use of SSR markers as a rapid and effective tool for identification and characterization of waxy corn hybrids along with their parental lines.

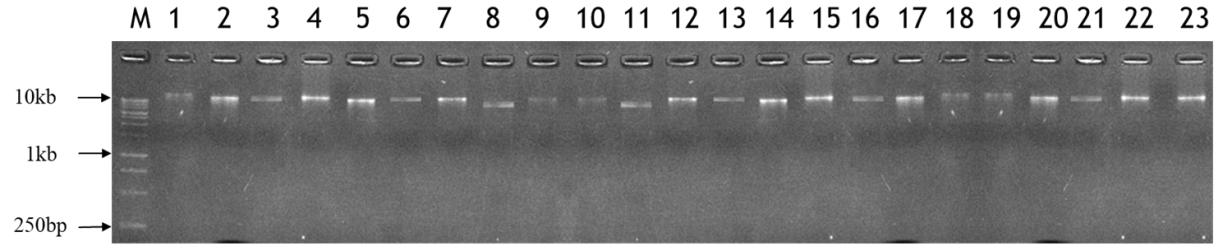

Figure 1. Single banding pattern obtained from all DNA samples extracted from waxy corn F1 hybrid, their parental lines and commercial hybrids. Lane M: 1kb marker, 1: BELLA 1-7, 2: BELLA 1-8, 3: BELLA 1-7 x BELLA 1-8, 4: BELLA 1-8 x BELLA 1-7, 5: BELLA 1-8, 6: BELLA 1-10. 7: BELLA 1-8 x BELLA 1-10, 8: BELLA 1-10 x BELLA 1-8, 9: BELLA 1-8, 10: BELLA 1-12. 11: BELLA 1-8 x BELLA 1-12, 12: BELLA 1-12 x BELLA 1-8, 13: BELLA 1-12, 14: GWT 46-1b. 15: BELLA 1-12 x GWT 46-1b, 16: GWT 46-1b x BELLA 1-12, 17: BELLA 1-12, 18: GWT 46-10b. 19: BELLA 1-12 x GWT 46-10b, 20: GWT 46-10b x BELLA 1-12, 21: C3, 22: C2, 23: C1.

A single banding pattern was observed from DNA extracted from waxy corn F1 hybrids, their parental lines, and commercial hybrids (Figure 1). Then, ten SSR primers (umc2366, bnlg2181, bnlg2162, umc1005, phi011, umc1196, umc2077, phi112, umc1153 and bnlg381) were screened by PCR amplification (Figure 2). Out of the ten SSR primers examined, only bnlg381 was able to generate a complimentary banding pattern of both parental lines, allowing for easy identification of their hybrid. The female parent (BELLA 1-8) produced a single band at 300bp, whereas the male parent (BELLA 1-7) produced a single band at 200bp. Thus, the hybrid BELLA 1-8 x BELLA 1-7 showed two co-dominant bands at 300bp and 200bp, confirming the heterozygosity and hybrid purity. In contrast, the hybrid BELLA 1-7 x BELLA 1-8 presented only one band at 200bp, as in the male parent (BELLA 1-7). Therefore, the bnlg381 and hybrid BELLA 1-8 x BELLA 1-7 were selected for further validation test. Moreover, Sivaranjani et al. (2014) and Zheng et al. (2013) also found that the primer bnlg381 was moderately informative in studying corn inbred lines.

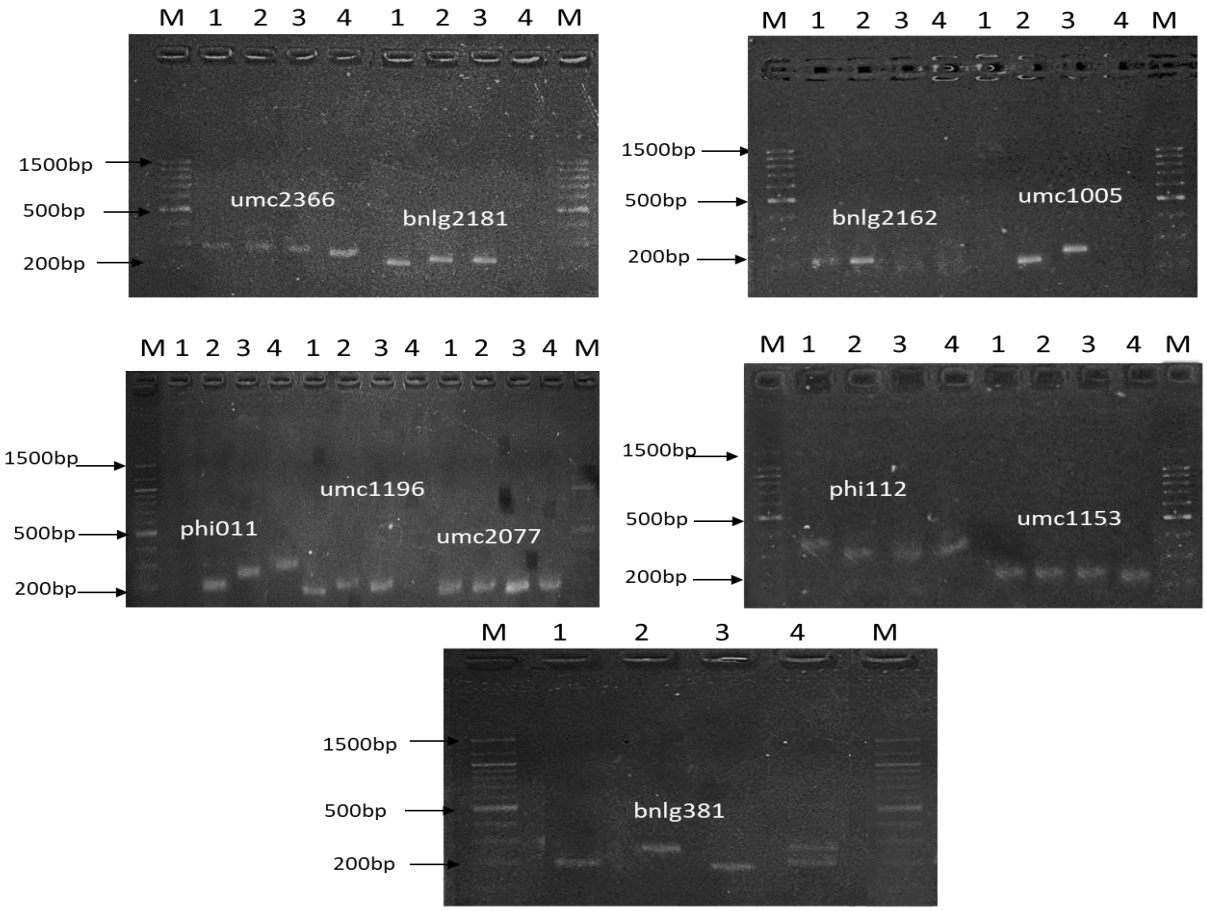

Figure 2. Banding pattern obtained through SSR-PCR amplifications of ten primers used in the study. Lane M: 1kb marker, 1: BELLA 1-7, 2: BELLA 1-8. 3: BELLA 1-7 x BELLA 1-8, 4: BELLA 1-8 x BELLA 1-7.

Validation of selected SSR markers for genetic purity testing of selected hybrid seeds

To validate the suitability of selected primer bnlg381, 20 randomly selected seeds of F1 hybrid BELLA 1-8 x BELLA 1-7 were tested for genetic purity. All samples showed that 100% seeds were genetically pure with the presence of complimentary bands from both their parents (Figure 3). This data suggested that there is no off-types or genotype mixing in the random sample collected from the seed lots of hybrid BELLA 1-8 x BELLA 1-7. This primer was successfully applied to distinguish the hybrids from their parental lines. Therefore, SSR marker is proved to be promising for identification, characterization, and selection of waxy corn hybrid seeds.

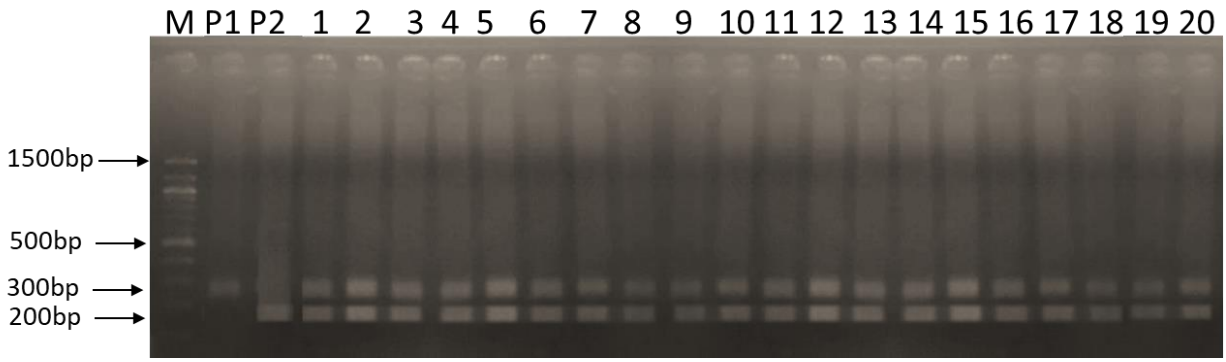

Figure 3. The primer bnlg381 confirming the genetic purity of 20 randomly selected hybrid seeds BELLA 1-8 x BELLA 1-7. Lane M: 100bp DNA ladder, P1: BELLA 1-8, P2: BELLA 1-7. 1-20: BELLA 1-8 x BELLA 1-7.

SSR markers have been used to assess hybrid genetic purity and molecular fingerprinting of parental lines in several crops, including rice (Bora et al., 2016; Cai et al., 2020), watermelon (Lu et al., 2018), cotton (Selvakumar et al., 2010), barley (Romdhane et al., 2018), and oriental melon (Nguyen et al., 2019). These studies emphasise the utility and effectiveness of SSR markers as an accurate and fast molecular tool for genetic purity testing, fingerprinting, and identification.

SSR-PCR analysis could be a useful method for hybrid purity testing, DNA fingerprinting for species identification and plant variety rights protection, as well as genome mapping and gene tagging (Williams et al., 1990). The use of molecular markers for genetic purity testing minimises the cost and time associated with selecting eligible plants for hybrid production and can be efficiently adopted by breeders (Tiwari et al., 2020). Due to its cost effectiveness, additional SSR marker analysis would be advantageous to the seed industry for routine evaluation of genetic purity of cultivars (Pattanaik et al., 2018). The information on SSR markers obtained from this work will be of great use to the hybrid waxy corn seed industry especially in selecting optimal SSR markers and evaluating the genetic purity of the plants at the seed stage.

CONCLUSION

In conclusion, ten SSR primers (umc2366, bnlg2181, bnlg2162, umc1005, phi011, umc1196, umc2077, phi112, umc1153 and bnlg381) were screened for waxy corn genetic purity testing. Out of ten primers, only one primer, bnlg381 produced complementary banding pattern of both parental lines, which made a way to identify the hybrid. The hybrid BELLA 1-8 x BELLA 1-7 has both DNA bands from its parents at 300bp and 200bp, confirming the genetic purity of this hybrid seed. The hybrid seed industry will benefit greatly from the SSR marker identified in this study, which will enable more cheaper and efficient selection of parental lines and evaluation of hybrid seeds in waxy corn. Hence, more SSR markers or other markers like random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), and restriction fragment length polymorphism (RFLP) need to be tested in further studies.

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REFERENCES

Bhat, M. I., Dar Gulzaffar, Z. A., Lone, A. A., Shikari, A. B., Ali, G., Wan, M. A., Khan, G. H., Gazal, A., & Lone, R. A. (2017). Genetic purity analysis in maize under temperate conditions. *International Journal of Current Microbiology and Applied Science, 6*(9), 2710-2722.

Bora, A., Choudhury, P. R., Pande, V., & Manda, A. B. (2016). Assessment of genetic purity in rice (*Oryza sativa* L.) hybrids using microsatellite markers. *3 Biotech, 6*(1), 50.

Cai, H., Lu, Y., Liu, G., Zhang, S., Jia, H., You, A., & Jiao, C. (2020). Genetic diversity analysis of hybrid rice parental lines and genetic purity assessment of hybrid seeds of China. *Journal of Agricultural Science*, *12*(5), 37-47.

Chaudhary, S., Dinesh, S., Prapati, D. R., Kharb, P., & Kamboj, M. C. (2018). Utilization of SSR markers for seed purity testing in popular maize hybrids. *International Journal of Current Microbiology Application and Applied Sciences, 7*(4), 1117-1126.

Chen, J. Y., Chen, Q. M., Liu, Z. G., Wang, C. L., Ma, L. L., Gou, J. Q., & Cheng, Z. K. (2020). Seed genetic purity testing of F1 *Benincasa hispida* var. *Chieh-qua* hybrids using SSR molecular marker analysis. *Seed Science and Technology, 48*(3), 345-353.

Devi, E. L., Hossain, F., Mthusamy. V., Chhabra, R., Zunjare, R. U., Baveja, A., Jaiswal, S. K., Goswami, R., & Dosad, S. (2017). Microsatellite marker-based characterization of waxy maize inbreds for their utilization in hybrid breeding. *3 Biotech*, *7*(5), 1-9.

Dong, L., Qi, X., Zhu, J., Liu, C., Z, X., Cheng, B., Mao, L., & Xie, C. (2019). Supersweet and waxy: meeting the diverse demands for specialty maize by genome editing. *Plant Biotechnology Journal*, *17,* 1853-1855.

Elci & Hancer. (2015). Genetic analysis of maize (*Zea mays* L.) hybrids using microsatellite marker. *Journal of Agricultural Science, 21*(2), 192-198.

Fernandez, E. C. J., Nuńez, J. P. P., Gardoce, R. R., Manohar A. N. C., Bajaro, R. M., & Lantican, D. V. (2023). Genetic purity and diversity assessment of parental corn inbred lines using SSR markers for Philippine hybrid breeding. *SABRAO Journal of Breeding and Genetics, 55*(3), 598-608.

Harakotr, B., Suriharn, B., Lertrat, K., & Scott, M. P. (2016). Genetic analysis of anthocyanin content in purple waxy corn (*Zea mays* L. var. *ceratina kulesh*) kernel and cob. *SABRAO Journal of Breeding and Genetics, 48*(2), 230-239.

Harakotr, H. R., Sa, K. J., Nam-Gung, M., Park, K. J., Ryu, S. H., Mo, C. Y., & Lee, J. K. (2021). Genetic characterization and association mapping in near-isogenic lines of waxy maize using seed characteristics and SSR markers. *Genes and Genomics, 43*(1), 79-90.

Hung, N. T., Huven, N. T., Van Loc, N., & Chuong, B. M. (2012). The application of SSR molecular indicator to assess the purity and genetic diversity of waxy inbred lines. *Journal of the International Society for Southeast Asian Agricultural Sciences, 18*(2), 45-54.

Kim, H. R., Sa, K. J., Nam-Gung, M., Park, K. J., Ryu, S. H., Mo, C. Y., & Lee, J. K. (2021). Genetic characterization and association mapping in near-isogenic lines of waxy maize using seed characteristics and SSR markers. *Genes and Genomics, 43*(1), 79-90.

Kovincic, A., Markovic, K., Ristic, D., Babic, V., Petrovic, T., Zivanovic, T., & Kravic, N. (2023). Efficiency of biological typing methods in maize hybrid genetic purity estimation. *Genes, 14*(6), 1195.

Kumar, R., Kumari, S., Singh, S. K., Singh, C. M., & Suman, S. K. (2022). Recent Advances in Rice Breeding Using Biotechnological and Genomics Tools. In U. Kamaluddin, Kiran, & M. Z. Abdin (Eds.), *Technologies in Plant Biotechnology and Breeding of Field Crops* (pp. 81-102). Springer Nature, Singapore.

Kiruthika, S., & Padmanabha, B. V. (2018). Determining genetic purity of commercial hybrids belonging to cucurbitaceous family using microsatellite markers. *International Journal of Creative Research Thoughts, 6*(2), 942-946.

Lu, X., Dedze, Y. M. N. A., Chofong, G. N., Gandeka, M., Deng, Z., Teng, L., Zhang, X., Sun, G., Si, L., & Li, W. (2018). Identification of high-efficiency SSR markers for assessing watermelon genetic purity. *Journal of Genetics, 97*(5), 1295-1306.

Luo, M., Shi, Y., Yang, Y., Zhao, Y., Zhang, Y., Shi, Y., Kong, M., Li, C., Feng, Z., Fan, Y., Xu, L., Xi, S., Lu, B., & Zhao, J. (2020). Sequence polymorphism of the waxy gene in waxy maize accessions and characterization of a new waxy allele. *Scientific Reports, 10*(1), 1-10.

Nguyen, N. N., Kwon, Y. S., Park, J. R., & Sim, S. C. (2019). Development of a core set of SSR markers for cultivar identification and seed purity tests in oriental melon (*Cucumis melo* L. var. *makuwa*). *Korean Journal of Horticultural Science and Technology, 37*(1), 119–129.

Padmanabha, B. V., & Kiruthika, S. (2018). Assessment of genetic purity of commercially cultivated hybrid vegetable crops of solanaceae family using SSR molecular markers. *International Research Journal of Engineering and Technology (IRJET), 5*(4), 735-738.

Pallavi, H. M., Gowda, R., Shadakshari, Y. G., Bhanuprakash, K., & Vishwanath, K. (2011). Identification of SSR markers for hybridity and seed genetic purity testing in sunflower (*Helianthus annuus* L.). *Helia, 34*(54), 59-66.

Pattanaik, A. D., Reddy, C. L., Ramesh, S., & Chennareddy, A. (2018). Comparison of traditional grow-out test and DNA-based PCR assay to estimate F1 hybrid purity in cauliflower. *Current Sciences, 115*(11), 2095-2102.

Romdhane, M. B., Riahi, L., Jardak, R., Ghorbel, A., & Zoghlami, N. (2018). Fingerprinting and genetic purity assessment of F1 barley hybrids and their salt-tolerant parental lines using n SSR molecular markers. *3 Biotech, 8*(1),57.

Sa, K., Park, J., Park, K., Lee, J. (2010). Analysis of genetic diversity and relationships among waxy maize inbred lines in Korea using SSR markers. *Genes Genomics, 32*(4), 375–84.

Selvakumar, P., Ravikesavan, R., Gopikrishnan, A., Thiyagu, K., Preetha, S., & Boopathi, N. M. (2010). Genetic purity analysis of cotton (*Gossypium* spp.) hybrids using SSR markers. *Seed Science and Technology, 38*, 358-366.

Sendekie, Y. (2020). Review on seed genetic purity for quality seed production. *International Journal of Scientific Engineering and Science, 4*(10) 1-7.

Shinde, N., Bharose, A., Sarode, D., Swathi, R., Pimpale, P., & Shinde, S. (2021). Assessment of hybrid purity in maize (*Zea mays* L.) using RAPD and SSR markers. *The Pharma Innovation Journal, 10*(4), 870-874.

Sivaranjani, R., Santha, I. M., Pandey, N., Vishwakarma, A. K., Nepolean, T., & Hossain, F. (2014). Microsatellite-based genetic diversity in selected exotic and indigenous maize (*Zea mays* L.) inbred lines differing in total kernel carotenoids. *Indian Journal of Genetics and Plant Breeding, 74*(1), 34.

Sudharani, M., Rao, P. S., & Subba Rao, L. V. (2012). Identification of SSR markers for testing of hybridity and seed genetic purity in maize (*Zea mays* L.). *International Journal of Science and Research (IJSR), 3*(10), 93-95.

Tiwari, S., Sao, S., Kurrey, A., & Das, P. (2020). Isolation and identification of molecular markers for fingerprinting of chilli hybrids & its parental lines. *International Journal of Research in Pharmaceutical Sciences, 11*(1), 713-716.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Afalski, A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research, 18*(22), 6531-6535.

Zheng, H., Wang, H., Yang, H., Wu, J., Shi, B., Cai, R., Xu, Y., Wu, A., & Luo, L. (2013). Genetic diversity and molecular evolution of Chinese waxy maize germplasm. *PLoS ONE, 8*(6), 19–22.

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