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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 1830-1835

www.thepharmajournal.com
Received: 03-10-2021
Accepted: 05-12-2021

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Characterization and differentiation of rice (*Oryza sativa* L.) Varieties through seed image analysis

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Abstract

The scope of morphological differences between the existing rice varieties is less due to narrow genetic base, and requires skilled human power which is subjective in nature. Also this process is time, labour and cost intensive. To overcome these practical problems an approach for fast and precise recognition of crop varieties is required. Keeping in view the above facts, the present study was initiated with the objective of characterization and differentiation of rice varieties through seed image analysis**.** Image analysis is a promising technique which can contribute significantly in improving the variety identification since it replaces the laborious manual inspection with cameras and image processing operations. This process uses digital images to measure the size of individual grains and mathematically extract features and shape related information from the images. In the present study, two different types of softwares were used for extraction of features from the images viz. Grain Analysis Software (for size and shape features) and MATLAB software (for textural features). The varieties (28) were grouped on the basis of these features generated from seed images. The study grouped the varieties into six clusters depicting better differentiation among the varieties on the basis of a single plant part i.e. seed through imaging pattern.

Keywords: Genetic variability, machine vision, clustering

Introduction

In Southeast Asia, rice is the staple food for 80% of the population (Ajay *et.al* 2013) [1]. In India, a systematic varietal improvement programme has led to the development of nearly 700 improved rice varieties, which are released and notified under the Seed Act, 1966. Even prior to this, many of the Agricultural Research Stations have been developing improved rice varieties for cultivation, which have now become a matter of common knowledge. There are about 950 released and notified varieties of rice (*Oryza sativa* L.) in India for which diagnostic features are well known and the same are followed for the purpose of seed certification. Hence, variety identification is of prime importance. However, under the PPV&FR Act, rice varieties are registered on the basis of Novelty, Distinctiveness, Uniformity and Stability (NDUS) characters which are taken as per the stage of observations listed in DUS guidelines (Chakrabarty *et al.* 2012) [2]. The scope of morphological differences between the varieties is less due to narrow genetic base, and requires skilled human power which is subjective in nature. Also this process is time, labour and cost intensive.

To overcome these practical problems an approach for fast and precise recognition of crop varieties is required. Image analysis is a promising technique which can contribute significantly in improving the variety identification since it replaces the laborious manual inspection with cameras and image processing operations. Digital image analysis offers an objective and quantitative method for estimation of morphological parameters. This process uses digital images to measure the size of individual grains and mathematically extract features and shape related information from the images (Shouche *et al.* 2001) [3].

Kaur and Verma (2013) [4] proposed computer vision technique for grading of rice kernels based on their sizes (full, medium, half). Singh and Banga (2012) [5] proposed image processing techniques for grading of rice samples based on their sizes.

Keeping in view the above facts, the present study was initiated with the objective of Characterization and differentiation of rice varieties through seed image analysis.

Materials and methods

A total of 28 basmati and non-basmati rice varieties were collected from different institutions and used for the experiment. With a view to realize the objectives enumerated in the introduction, the laboratory experiments were carried out in the Division of Seed Science and Technology, Indian Agriculture Research Institute New Delhi.

Imaging Protocol

Scanner- The images of seed were taken by using flatbed scanner (Canon Lide 110 Version 1.2.00) at resolution power of 600 dpi. Ten seeds in three replication of each variety were taken and placed on scanner by avoiding grain to grain contact. The images were saved in. jpeg format for further processing.

Image Processing and extraction of features

The image processing was done by using two different kinds of software's:

- Grain Analysis Software developed by Dr. Nachiket Kotwaliwale.
- Used for extraction of size and shape features from the seed
- MATLAB software (version 7.12.0.635,R2011a) developed by Dr. Nachiket.
- Used for extraction of textural features from the seed and leaf images.

Eleven parameters measured from the Grain Analysis Software: Area, Perimeter, Bounding box length, Bounding box width, Axial length, Axial width, Median length, Median width, Eccentricity, Roundness, Equivalent diameter.

The basic features recorded by the MATLAB software

1. **Morphological features:** Length, Width, Awn length, Kernel area, Kernel perimeter, Major axis, Minor axis, Eccentricity, Equivalent Diameter, Length-width ratio
2. **Textural features:** Contrast; Correlation; Energy; Homogeneity; Range; STD; Entropy; Offset 0; Offset 45; Offset 90; Offset 135; SRE; LRE; GLN; LP; RLN; LGRE; HGRE

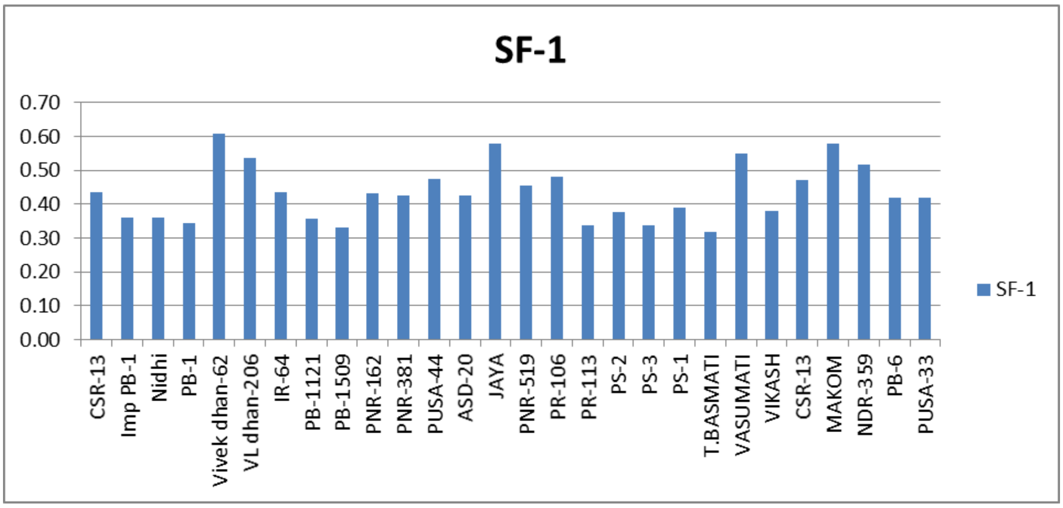
Chromatic features: Redness; Greenness; Blueness; Hue; Saturation; Value; Hue Std; RHS colour value

Table 1: Seed data generated by Grain analysis software

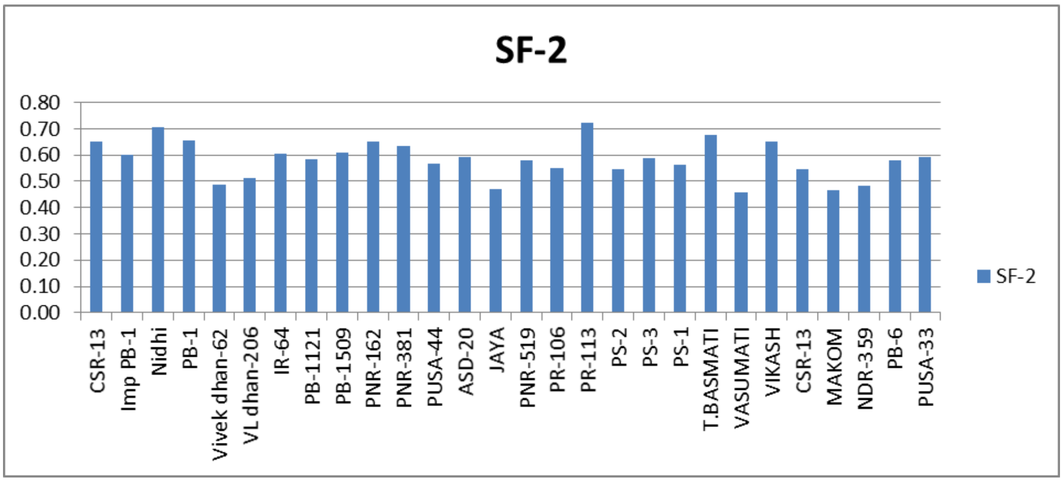
S. No.	VAR	Area, mm^2	Length, mm	Breadth, mm	Eccentricity	Perimeter, mm	Equivalent Dia, mm	Roundness	Axial Length, mm	Axial Width, mm	Median Length, mm	Median Width, mm
1	CSR-13	13.421	8.74	2.1	0.973	19.66	4.13	0.2227	4.8	1.98	3.5221	1.66
2	Imp PB-1	19.912	11.93	2.49	0.98	26.4	5.04	0.1782	6.35	2.26	5.0165	1.9
3	Nidhi	14.278	10.1	2.06	0.979	22.3	4.26	0.1791	5.03	1.83	4.0131	1.56
4	PB-1	17.636	11.55	2.17	0.982	25.44	4.73	0.1678	7.18	1.97	4.8301	1.66
5	Vivek dhan-62	15.768	7.71	2.79	0.931	18.07	4.48	0.3386	3.94	2.64	2.7646	2.31
6	VL dhan-206	17.09	8.74	2.69	0.948	20.01	4.66	0.2867	3.74	2.58	2.6946	2.19
7	IR-64	15.538	9.41	2.33	0.97	21.16	4.42	0.223	7.37	2.09	3.9475	1.81
8	PB-1121	21.639	12.65	2.39	0.985	27.6	5.25	0.1719	5.61	2.31	3.2006	1.85
9	PB-1509	21.783	13.25	2.31	0.984	28.76	5.24	0.1592	7.82	2.23	4.6059	1.88
10	PNR-162	13.658	8.92	2.16	0.971	19.97	4.18	0.2199	4.32	2	3.086	1.66
11	PNR-381	14.776	9.36	2.13	0.975	20.86	4.34	0.2156	4.13	2.04	3.1412	1.7
12	PUSA-44	16.009	9.05	2.34	0.967	20.58	4.5	0.249	5.59	2.22	4.1274	1.93
13	ASD-20	16.574	9.82	2.37	0.973	22.12	4.6	0.2197	5.63	2.12	4.6057	1.85
14	JAYA	17.848	8.42	2.91	0.937	19.68	4.75	0.3214	4.12	2.8	2.9294	2.38
15	PNR-519	16.108	9.33	2.32	0.968	21.13	4.52	0.236	7.36	2.17	4.6354	1.94
16	PR-106	16.64	9.15	2.53	0.961	20.87	4.6	0.2544	6.28	2.36	3.884	2
17	PR-113	14.678	10.6	2.09	0.981	23.43	4.3	0.1663	6.49	1.86	5.4567	1.51
18	PS-2	22.663	12.38	2.61	0.978	27.53	5.37	0.1896	10.05	2.37	5.3637	2.08
19	PS-3	21.907	12.9	2.52	0.984	28.52	5.26	0.1679	9.66	2.23	5.9139	1.92
20	PS-1	20.841	11.75	2.41	0.977	25.96	5.15	0.1928	6.39	2.23	5.2747	2.01
21	T.BASMATI	17.584	11.88	2.42	0.981	26.33	4.72	0.1587	8.77	2.13	4.119	1.67
22	VASUMATI	20.312	9.29	2.9	0.949	21.53	5.08	0.2989	5.31	2.77	3.7762	2.42
23	VIKASH	15.836	10.31	2.31	0.975	22.89	4.48	0.1893	5.6	2.05	4.2122	1.71
24	CSR-13	17.601	9.58	2.47	0.966	21.69	4.73	0.2436	5.63	2.32	4.5847	2.02
25	MAKOM	18.23	8.52	2.84	0.94	19.91	4.81	0.3202	4.66	2.72	3.3974	2.39
26	NDR-359	19.732	9.54	2.78	0.955	21.88	5.01	0.2769	4.68	2.65	3.6577	2.28
27	PB-6	17.464	10.13	2.34	0.974	22.9	4.71	0.216	6.66	2.2	5.1096	1.89
28	PUSA-33	17.173	10.2	2.35	0.974	22.67	4.68	0.2112	5.29	2.18	3.446	1.85

Shape Factors based on Feature extraction of Grains

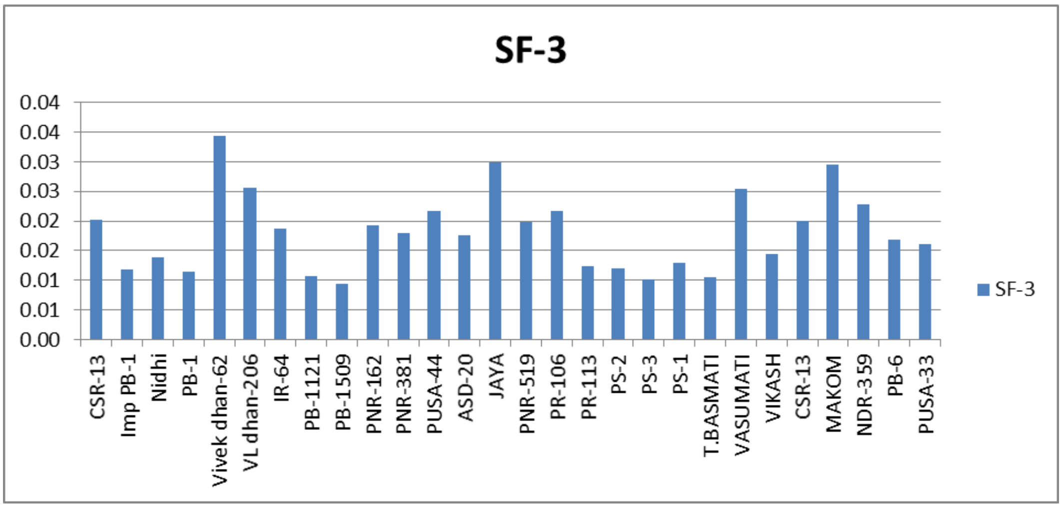
From the values of axis length, perimeter and area, shape factors were derived, following Symons and Fulcher (1988) formulae.



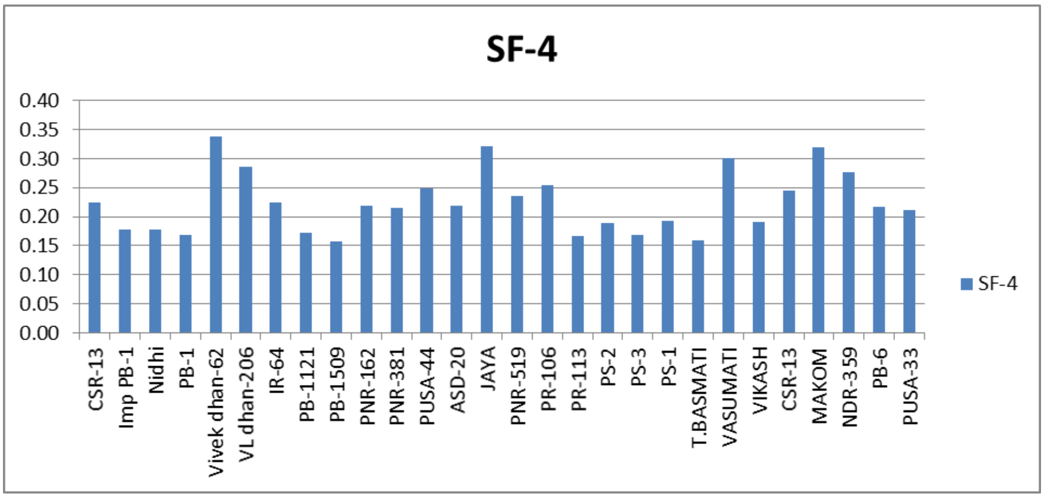
Shape factor 1: 4 π Area/Perimeter²



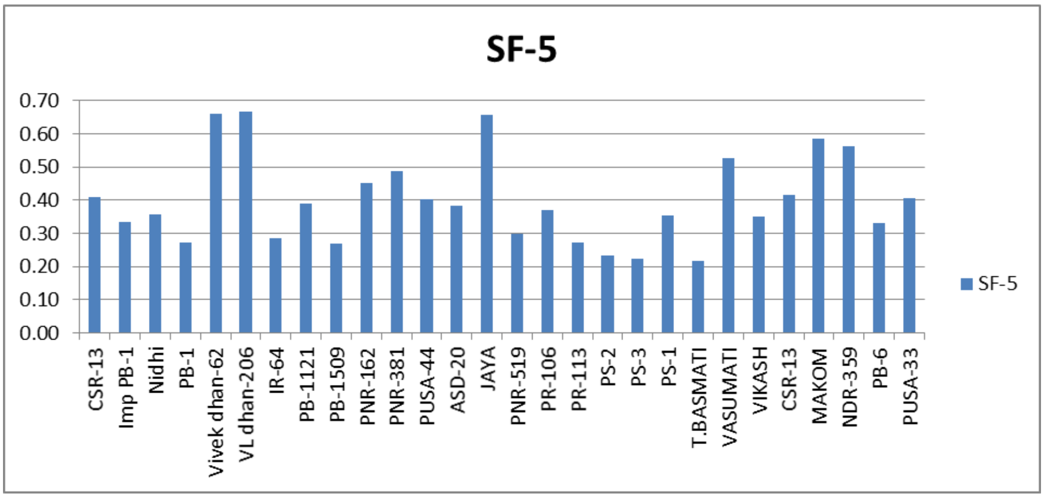
Shape factor 2: Major axis length/Area



Shape factor 3: Area/Major axis length³



Shape factor 4: Area/(Major axis length/2)(Major axis length/2)



Shape factor 5: Area/(Major axis length/2)(Minor axis length/2)

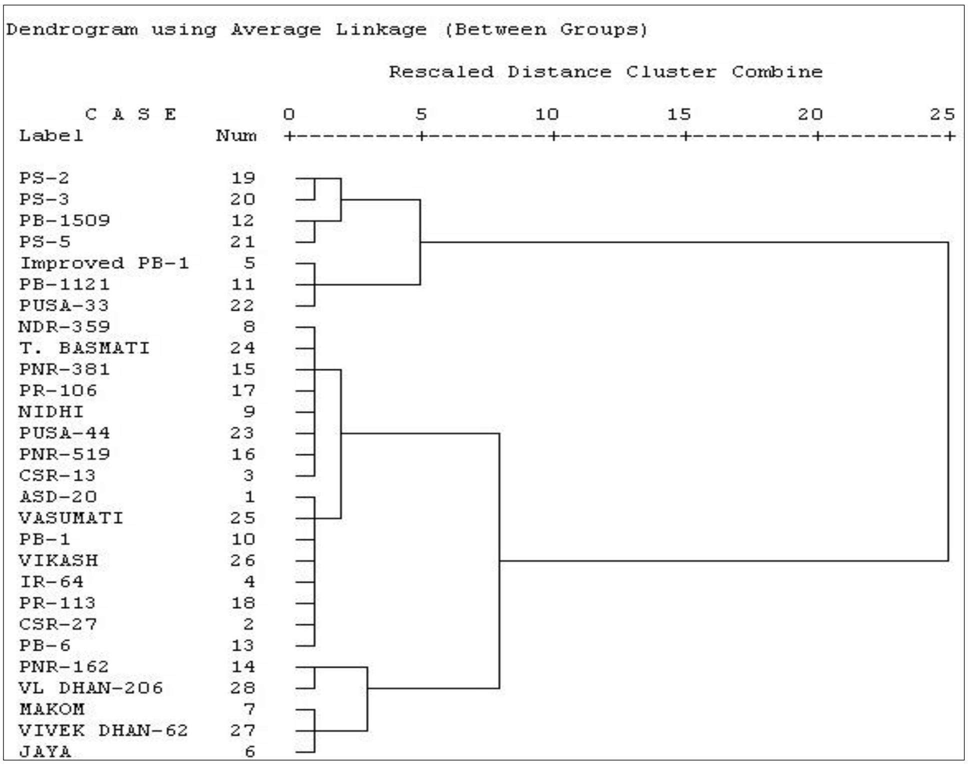


Fig 1: Dendrogram generation based on Seed data generated through MATLAB software

Table 2: Clustering pattern of varieties based on Seed data generated through MATLAB software

Cluster	Number of varieties	Variety Name
I	4	PS-2, PS-3, PB-1509, PS-5,
II	3	IMPROVED PB-1, PB-1121, PUSA-33
III	8	NDR-359, TARAORI BASMATI, PNR-381, PR-106, NIDHI, PUSA-44, PNR-519, CSR-13
IV	8	ASD-20, VASUMATI, PB-1, VIKASH, IR-64, PR-113, CSR-27, PB-6
V	2	PNR-162, VL DHAN-206,
VI	3	MAKOM, VIVEK DHAN-62, JAYA

Result and Discussion

The present study was initiated with the objective of Characterization and differentiation of rice varieties through seed image analysis**.** With this regard, for seed imaging, flatbed scanner Canon LiDE 110 version 1.2.00 was used to develop the image library. Ten rice grains for each variety were placed on scanner avoiding grain to grain contact. The images were taken in three replications per variety. That accounted for a total of 28 x 3 = 84 images; with an average of 10 seeds per image. Images of all grains were grabbed at resolution of 600 dpi. The images were stored in. jpeg *format* for further analysis. Grain Analysis Software (developed by Nachiket Kotwaliwale, CIAE, Bhopal) was used for further processing of the seed images.

The seed data generated by the Grain Analysis Software is presented in Table 1. Table 1 shows the average results for samples of 10 grains of one cultivar, computed over three replications, for all the 28 varieties. The data for three replications showed comparable values for all the parameters. This demonstrated the accuracy of the scanner for digital inputs of the image attributes. Average values of the area, perimeter, bounding box length, bounding box breadth, axial length, axial width, median length, median width, eccentricity, equivalent diameter, and roundness are presented in mm, using the calibration factor of the scanned image. Data on mean, standard error (S.E) and coefficient of variation (C.V) of all the parameters is also presented alongside

A perusal of the various shape factors revealed that varieties T. Basmati and Vivek Dhan 62 were significantly different from each other for Shape factor 1, varieties Vasumati and PR 113 for Shape factor 2, varieties PB 1509 and Vivek Dhan 62 for Shape factors 3 and 4, and T. Basmati and Jaya were significantly different from each other for Shape factor 5. A comparative assessment of various shape factors revealed that shape factor 5 involving area and major and minor axis length was most useful for distinguishing varieties.

Textural features extracted from Seed images

The 28 varieties were found to differ significantly from each other with respect to these textural features. Since these features were exclusively related to the seed images only, hence an attempt was made to classify/ group the varieties on the basis of features extracted from the seed images. The dendrogram depicting the classification of these varieties is depicted in Fig. 1 and the clustering pattern of the varieties is given in Table 2. The study grouped the varieties into six clusters depicting better differentiation among the varieties on the basis of a single plant part i.e. seed through imaging pattern.

Similar study was conducted to analyze the shapes of brown rice and polished rice, a series of measurements by image processing on Japonica, Indica and Javanica types composed of four rice varieties with three polishing methods were carried out. Area, perimeter; maximum length, maximum width, compactness and elongation were measured. Further; separating the rice varieties by the shape difference of a rice particle was examined. From the result of the investigation on brown rice and polished rice, separating the rice varieties was possible at a probability level of 95.45% with combined dimensions and shape factors or with single ones (N. Sakai, *et al*. 1996) [6].

In present study the size and shape features extracted by the Grain Analysis software as well as derived shape factors clearly distinguished the varieties into various clusters. The additional textural features extracted from seed images (about 27 textural features) further aided in the differentiation of varieties.

Zhu, F., *et al.* (2021) [7] developed an open-source graphical user interface (GUI) software, Seed Extractor that determines seed size and shape (including area, perimeter, length, width, circularity, and centroid), and seed color with capability to process a large number of images in a time-efficient manner and identified known loci for regulating seed length (*GS3*) and width (*qSW5/GW5*) in rice, which demonstrates the accuracy of this application to extract seed phenotypes and accelerate trait discovery. Vale, A *et al.*, (2020) [8] presented. Open source plugin for the automatic segmentation of an image of a seed sample. The new plugin was tested on a total of 3,386 seed samples from 120 species belonging to the Fabaceae family. Digital images were acquired using a flatbed scanner. The results showed that the new plugin was able to segment all of the digital images without generating any object detection errors. In addition, the new plugin was able to segment images within an average of 0.02 s, while the average time for execution with the manual method was 63 s.

Sarigu, M *et al.* (2019) [9] studied 124 morpho colorimetric quantitative and qualitative features of seeds of the *Paeonia mascula* group from the Balearic Islands, Corsica, Sicily, and Sardinia were measured by an image analysis system to evaluate whether differences in seed morphology and results showed that the seeds of the studied taxa were distinguishable with a high percentage of classification and highlighted that three different taxa are identifiable in Corsica (*P. corsica*, *P. mascula* subsp. *mascula*, and *P. morisii*), while only one (*P. cambessedesii*) is identifiable in the Balearic Islands.

Sau, S *et al.* (2018) [10] used. Seed image analysis to discriminate apple germplasm accessions. Digital images of seeds from 42 apple cultivars, acquired by a flatbed scanner, provided a phenotypic dataset with 106 morphometric variables. Stepwise Linear Discriminant Analysis (LDA) was used to examine this dataset, and the results were compared with available genetic data. In agreement with the genetic diversity analysis, the LDA could discriminate between the apples cultivars, identifying two main groups that could be further divided into additional subgroups.

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