Vol 5 | Issue 1 | Pages 384-389
Journal of Rice Research and Developments
ISSN: 2643-5705

Research Article
DOI: 10.36959/973/437

# Euclidean Distance Detects Morphoagronomic Differences in the Progeny of Rice Regenerated Plants Compared to the Traditional Cultivar

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## Abstract

*In vitro* culture is recognized as one of the main causes of phenotypic, morphological or biochemical differences of vitroplants compared to the traditional cultivars. The Euclidean distance algorithm could be an easy and useful tool for analyzing the morphological variations between both types of plants. In the present work this algorithm was used for the simultaneous evaluation of some morphoagronomic parameters in the progeny of transgenic and non-transgenic rice plants (cv.Jucarito-104) regenerated from embryogenic calli, with respect to a control group of plants of Cuban traditional cultivar Jucarito-104. All plants were grown in a grow house with environmental conditions similar to those of rice fields. The evaluated parameters were: panicle length, dry weight of 1000 seeds, seeds per panicle, seed length, seed width and length/width ratio. The calculation of Euclidean distance allowed detecting differences between the plants from the traditional cultivar and the progeny of *in vitro* plants, whether or not they were transgenic. The similar behavior of both *in vitro* progenies suggested that morphoagronomic variations are due to *in vitro* culture, which is the condition they have in common. The application of the Euclidean distance provided evidences about the occurrence of changes in morphoagronomic parameters in plants obtained *in vitro*, and it also could contribute to evaluate the phenotypic fidelity of transgenic plants to the traditional cultivar.

## Keywords

*In vitro* culture, Regenerated plants, Somaclonal variation, Transgenic rice

## Introduction

*In vitro* culture is recognized as one of the main causes of genomic changes in plants. There are frequent reports that the plants obtained *in vitro* show phenotypic, morphological or biochemical differences, compared to the donor of the explants. This phenomenon is known as somaclonal variation and is associated with chromosomal recombination, random mutations, and changes in the ploidy level that cause genetic instability [1]. Somaclonal variation has important implications for breeding programs, but it can be undesirable when required to maintain the clonal identity of the cultured material. The main factors that induce *in vitro* somaclonal variations are the genotype, the chimeric nature of the explant, the culture conditions and the maintenance of the *in vitro* culture of the plant material for a long time [2].

Genetic transformation generally involves long stages of *in vitro* culture and selection of the transformed material. In the genetic transformation of the Cuban traditional rice cultivar Jucarito-104, the initial explant that has been used is the callus [3], which is the explant par excellence to induce somaclonal variations, since it is induced in a culture medium with 2.4-D, a mutagenic agent. After the step of genetic transformation, where a foreign gene is incorporated by the biolistic method, the calli are subjected to a selection pressure with antibiotics and are kept for thirty days in the culture medium with 2.4-D, until they are transferred to a regeneration medium with strong auxins and with an antibiotic for the selection of transformants during two months [3]. Therefore, it is probable that the lines that are obtained as a result of this long process present phenotypic differences compared to plants that have not been cultivated *in vitro*.

It would be convenient to demonstrate whether or not there are phenotypic differences between plants obtained by

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**Accepted:** May 27, 2022

**Published online:** May 29, 2022

**Citation:** Pérez-Bernal M, Delgado M, Abreu D, et al. (2022) Euclidean Distance Detects Morphoagronomic Differences in the Progeny of Rice Regenerated Plants Compared to the Traditional Cultivar. J Rice Res Dev 5(1):384-389

*in vitro* culture and the plants from traditional cultivar. The Euclidean distance algorithm could be functional for this type of study, since it is suitable to determine the similarity between pairs of data. This algorithm is the application of a mathematical formula for measuring the distance in a straight line between two points in an n-dimensional space and responds to the concept of distance as the hypotenuse of a right triangle [4]. In plant biotechnology it has been used for the concurrent selection of some agricultural characters [5] and to analyze the morphological diversity between several important cultivars such as cassava [6], pineapple [7] and in the case of rice to compare germplasms from different origin and varietal group [8], but it never has been applied for the evaluation of morphological changes that can occur in rice plants from *in vitro* culture.

The objective of this work was to evaluate simultaneously, using the Euclidean distance algorithm, some morphoagronomic parameters in the maturation stage of rice plants from the progeny of transgenic and non-transgenic plants (cv. Jucarito-104), regenerated from embryogenic calli, with respect to plants from the traditional cultivar.

## Materials and Methods

### Groups of plants

Three groups of rice plants were cultivated in a grow house protected by bird netting. Environmental conditions in the grow house were similar to those of rice fields in terms of type of soil, temperature, humidity, water supply and sunlight.

Group 1: Transgenic plants regenerated from embryogenic calli, induced from J-104 rice seeds, transformed by biolistic method, harboring the defensin gene *NmDef02* [3].

Group 2: Plants regenerated from non-transformed embryogenic calli, also induced from J-104 rice seeds.

The regeneration of the plants included in both groups was performed by the procedure described by Pérez-Bernal and colleagues [9]. After the plantlets reached 10-15 cm height they were transferred to *ex vitro* environment in the grow house.

Group 3 (control group): Plants of the traditional rice cultivar Jucarito-104.

The plants germinated from the seeds obtained from the three groups of plants were also cultivated in the grow house until the maturation stage. Thirty plants per group were randomly selected for the evaluation of morphoagronomic parameters.

### Morphoagronomic parameters

The morphoagronomic parameters evaluated in the maturation stage for each group of plants were: the panicle length (cm), the dry weight of 1000 seeds (g), the number of seeds per panicle, the seed length (mm), the seed width (mm) and the length/width ratio. These parameters were compared with those reported by the experts for the Cuban traditional cultivar Jucarito-104 [10], which are presented in Table 1.

The Euclidean distance was applied for the simultaneous evaluation of the morphoagronomic parameters, using the expert criteria for the cultivar Jucarito-104. The formula to calculate the Euclidean distance (ED) was as follows:

$$ED = \sqrt{(PL-26.7)^2 + (DW-31)^2 + (SP-127)^2 + (SL-9.38)^2 + (SW-2.6)^2 + (L/W-3.6)^2}$$

Where:

PL: Panicle Length (cm); DW: Dry Weight of 1000 Seeds (g); SP: Seeds per Panicle; SL: Seed Length (mm); SW: Seed Width (mm); L/W: Length/Width Ratio

## Results and Discussion

The results of the measurements of the morphoagronomic parameters in the maturation stage of the studied plants are presented in the Table 2, Table 3 and Table 4. Half of the control plants had a value of the Euclidean distance equal to zero, which means that there were no deviations in the measurements of the parameters under study. Null Euclidean distance signified the ideal morphological status. In the rest of these plants, the values were in the range between 0.02 and 2.24 (Table

**Table 1:** Morphoagronomic parameters described by experts for the Cuban traditional rice cultivar Jucarito-104 in the maturation stage.

| Morphoagronomic parameters | Expert criteria |
| --- | --- |
| Panicle length | 26.7 cm |
| Dry weight of 1000 seeds | 31.0 g |
| Seeds per panicle | 127 |
| Seed length | 9.38 mm |
| Seed width | 2.6 mm |
| Length/width ratio | 3.60 |

**Table 2:** Evaluation of morphoagronomic parameters in the maturation stage of transgenic rice plants (var. Jucarito-104).

| Plants | PL (cm) | DW (g) | SP | SL (mm) | SW (mm) | L/W | ED |
|---|---|---|---|---|---|---|---|
| 1 | 23.23 | 29 | 91 | 9.21 | 2.90 | 3.17 | 47.63 |
| 2 | 22.55 | 29 | 85 | 9.45 | 2.86 | 3.30 | 42.25 |
| 3 | 22.74 | 29 | 89 | 9.05 | 2.85 | 3.17 | 38.26 |
| 4 | 21.81 | 30 | 96 | 9.28 | 2.65 | 3.50 | 31.40 |
| 5 | 21.66 | 30 | 68 | 9.75 | 2.78 | 3.50 | 59.22 |
| 6 | 22.25 | 30 | 70 | 9.61 | 2.81 | 3.42 | 57.18 |
| 7 | 22.03 | 29 | 73 | 9.33 | 2.77 | 3.37 | 54.23 |
| 8 | 26.40 | 31 | 130 | 9.38 | 2.63 | 3.56 | 3.01 |
| 9 | 23.40 | 30 | 92 | 9.61 | 2.80 | 3.43 | 35.17 |
| 10 | 21.76 | 29 | 81 | 8.98 | 2.87 | 3.13 | 46.31 |
| 11 | 26.23 | 30 | 127 | 9.35 | 2.63 | 3.55 | 4.80 |
| 12 | 22.23 | 30 | 107 | 9.53 | 2.41 | 3.95 | 20.52 |
| 13 | 20.90 | 32 | 88 | 9.56 | 2.96 | 3.23 | 39.44 |
| 14 | 23.81 | 30 | 107 | 9.42 | 2.87 | 3.28 | 20.23 |
| 15 | 24.50 | 29 | 89 | 9.54 | 2.84 | 3.36 | 38.12 |
| 16 | 23.66 | 29 | 84 | 9.30 | 2.72 | 3.42 | 43.15 |
| 17 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 18 | 26.03 | 31 | 128 | 9.48 | 2.67 | 3.55 | 1.21 |
| 19 | 23.37 | 28 | 96 | 9.10 | 2.82 | 3.23 | 31.33 |
| 20 | 23.65 | 28 | 84 | 9.22 | 2.82 | 3.27 | 43.21 |
| 21 | 22.34 | 31 | 73 | 9.30 | 2.81 | 3.31 | 54.10 |
| 22 | 26.70 | 31 | 129 | 9.38 | 2.61 | 3.59 | 2.00 |
| 23 | 22.94 | 29 | 89 | 9.33 | 2.90 | 3.22 | 38.24 |
| 24 | 23.70 | 29 | 90 | 9.10 | 2.77 | 3.28 | 37.19 |
| 25 | 22.30 | 29 | 82 | 9.37 | 2.85 | 3.29 | 45.26 |
| 26 | 21.65 | 29 | 98 | 9.06 | 2.78 | 3.26 | 29.51 |
| 27 | 23.00 | 31 | 96 | 9.45 | 2.91 | 3.25 | 31.23 |
| 28 | 26.78 | 30 | 126 | 9.38 | 2.83 | 3.31 | 1.46 |
| 29 | 22.10 | 30 | 104 | 9.45 | 2.60 | 3.30 | 23.48 |
| 30 | 26.81 | 31 | 127 | 9.39 | 2.62 | 3.58 | 0.11 |

PL: Panicle Length; DW: Dry Weight of 1000 Seeds; SP: Seeds per Panicle; SL: Seed Length; SW: Seed Width; L/W: Length/Width Ratio; ED: Euclidean Distance

4). Thus, the calculation of the Euclidean distance revealed that the environmental conditions in the grow house affected slightly the morphoagronomic parameters of the control group, since these conditions were similar to those founded in the rice fields. Köhl [11] has referred the difficult cultivation of rice outside the rice production area, but, according to our results, the grow house environment allowed the success compliment of the life cycle of the cultivar J-104, for the water and micronutrient availability and mainly for the light demand, since this cultivar makes better use of sunlight due to the position presented by the angle of its leaves. Therefore, a correct cultivation technique was used and the results of the evaluation of the morphoagronomic parameters in all the plants under study may be similar to those obtained in the rice fields.

The maximum value of Euclidean distance in the control plants, 2.24, was taken as the cut-point of the assessment. The plants from *in vitro* progeny with Euclidean distance values higher than the cut-point were considered morphologically different with respect to the traditional variety. Taking this criterion into account, the vast majority of the plants from the progeny of transgenic and non-transgenic plants regenerated from callus (groups 1 and 2) had changes in their morphology compared to J-104 original cultivar. The Euclidean distance values were upper than 20 in 76% of the plants from both groups (Table 2 and Table 3); they presented marked phenotypic differences with respect to the traditional cultivar. The most notable change was found in the number of seeds per panicle, with an average of 97.53 in the transgenic plants and 102.73 in the non-transgenics, distant from the

**Table 3:** Evaluation of morphoagronomic parameters in the maturation stage of rice plants (cv. Jucarito-104) regenerated from non-transformed calli.

| Plants | PL (cm) | DW (g) | SP | SL (mm) | SW (mm) | L/W | ED |
|---|---|---|---|---|---|---|---|
| 1 | 24.81 | 27 | 102 | 9.10 | 2.76 | 3.29 | 23.42 |
| 2 | 20.42 | 30 | 72 | 9.72 | 2.86 | 3.40 | 55.36 |
| 3 | 23.37 | 29 | 88 | 9.30 | 2.86 | 3.25 | 39.19 |
| 4 | 26.78 | 30 | 126 | 9.38 | 2.83 | 3.31 | 1.46 |
| 5 | 21.60 | 32 | 75 | 9.63 | 2.86 | 3.37 | 52.26 |
| 6 | 24.11 | 29 | 143 | 9.50 | 2.75 | 3.45 | 16.33 |
| 7 | 23.81 | 30 | 123 | 9.32 | 2.87 | 3.43 | 19.23 |
| 8 | 23.00 | 30 | 129 | 9.61 | 2.80 | 3.43 | 25.17 |
| 9 | 25.10 | 31 | 129 | 9.56 | 2.66 | 3.59 | 13.15 |
| 10 | 26.60 | 31 | 126 | 9.33 | 2.61 | 3.57 | 4.47 |
| 11 | 26.07 | 31 | 124 | 9.39 | 2.60 | 3.61 | 3.46 |
| 12 | 25.93 | 31 | 129 | 9.33 | 2.60 | 3.58 | 2.15 |
| 13 | 20.90 | 32 | 88 | 9.56 | 2.96 | 3.23 | 39.44 |
| 14 | 23.66 | 29 | 84 | 9.30 | 2.72 | 3.42 | 43.15 |
| 15 | 24.50 | 29 | 89 | 9.54 | 2.84 | 3.36 | 38.12 |
| 16 | 21.65 | 29 | 98 | 9.06 | 2.78 | 3.42 | 29.50 |
| 17 | 23.23 | 29 | 91 | 9.22 | 2.89 | 3.17 | 47.64 |
| 18 | 22.25 | 30 | 69 | 9.60 | 2.81 | 3.42 | 57.19 |
| 19 | 23.37 | 28 | 96 | 9.10 | 2.82 | 3.23 | 31.33 |
| 20 | 21.81 | 29 | 96 | 9.27 | 2.64 | 3.50 | 31.41 |
| 21 | 22.55 | 29 | 85 | 9.40 | 2.85 | 3.31 | 42.20 |
| 22 | 26.71 | 31 | 128 | 9.36 | 2.68 | 3.49 | 1.00 |
| 23 | 22.90 | 27 | 89 | 9.32 | 2.90 | 3.20 | 38.20 |
| 24 | 23.71 | 26 | 93 | 9.11 | 2.80 | 3.26 | 40.10 |
| 25 | 22.30 | 29 | 82 | 9.37 | 2.85 | 3.29 | 45.26 |
| 26 | 26.81 | 31 | 127 | 9.39 | 2.62 | 3.58 | 0.11 |
| 27 | 26.69 | 31 | 128 | 9.36 | 2.68 | 3.49 | 1.00 |
| 28 | 22.03 | 30 | 73 | 9.32 | 2.77 | 3.38 | 54.25 |
| 29 | 23.40 | 30 | 94 | 9.60 | 2.80 | 3.42 | 35.22 |
| 30 | 22.20 | 30 | 106 | 9.52 | 2.77 | 3.43 | 21.52 |

PL: Panicle Length; DW: Dry Weight of 1000 Seeds; SP: Seeds per Panicle; SL: Seed Length; SW: Seed Width; L/W: Length/Width Ratio; ED: Euclidean Distance

127 seeds per panicle reported for the traditional cultivar. In both groups of plants regenerated *in vitro*, the panicles were shorter than the panicles of the traditional cultivar, with an average of 23.5 cm and 23.7 cm in the transgenic and non-transgenic, respectively. Regarding the dry weight of 1000 seeds, a weight of 31 grams has been reported for the traditional cultivar; however, this weight was reduced in the seeds from the others two groups of plants: 1000 dry seeds obtained from the transgenic plants weighed an average of 29.80 grams, and from the non-transgenic plants, 29.66 grams. Only the plant number 17 of the transgenic group fulfilled all the morphoagronomic parameters defined by the experts for the traditional cultivar. The Euclidean distance in this case was zero (Table 2).

In both progenies of plants from *in vitro* culture, the Euclidean distance values allowed the detection of changes in the morphoagronomic parameters with respect to the control group, suggesting that these variations are not due to transgenesis but to *in vitro* culture, which is the condition they have in common. These plants, transgenic or not, were descendants from plants regenerated from embryogenic calli induced on culture medium supplemented with 2.4-D. The negative cytogenetic effects of 2.4-D have been demonstrated in plant tissue culture, which can affect the genetic purity of the treated material [12]. Moreover, the calli were incubated for two months in a culture medium with kinetin and two strong auxins for the plant regeneration. These regeneration conditions also contribute to the occurrence of somaclonal

**Table 4:** Evaluation of morphoagronomic parameters in the maturation stage of rice plants of traditional rice cultivar Jucarito-104.

| Plants | PL (cm) | DW (g) | SP | SL (mm) | SW (mm) | L/W | ED |
|---|---|---|---|---|---|---|---|
| 1 | 26.70 | 30 | 128 | 9.38 | 2.62 | 3.58 | 1.41 |
| 2 | 26.71 | 31 | 127 | 9.36 | 2.60 | 3.60 | 0.02 |
| 3 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 4 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 5 | 26.70 | 30 | 125 | 9.32 | 2.61 | 3.57 | 2.24 |
| 6 | 26.71 | 31 | 126 | 9.38 | 2.63 | 3.57 | 1.00 |
| 7 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 8 | 26.68 | 31 | 127 | 9.38 | 2.64 | 3.55 | 0.06 |
| 9 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 10 | 26.63 | 31 | 127 | 9.37 | 2.60 | 3.60 | 0.07 |
| 11 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 12 | 26.63 | 31 | 127 | 9.37 | 2.62 | 3.58 | 0.08 |
| 13 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 14 | 26.70 | 31 | 127 | 9.38 | 2.63 | 3.57 | 0.04 |
| 15 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 16 | 26.72 | 32 | 129 | 9.38 | 2.60 | 3.61 | 2.24 |
| 17 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 18 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 19 | 26.71 | 30 | 127 | 9.37 | 2.60 | 3.61 | 1.00 |
| 20 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 21 | 26.68 | 31 | 129 | 9.38 | 2.60 | 3.61 | 2.00 |
| 22 | 26.68 | 31 | 126 | 9.35 | 2.62 | 3.57 | 1.00 |
| 23 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 24 | 26.70 | 30 | 127 | 9.38 | 2.60 | 3.61 | 1.00 |
| 25 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 26 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 27 | 26.72 | 32 | 129 | 9.38 | 2.60 | 3.61 | 2.24 |
| 28 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 29 | 26.70 | 31 | 127 | 9.38 | 2.60 | 3.60 | 0.00 |
| 30 | 26.72 | 32 | 127 | 9.37 | 2.62 | 3.58 | 1.00 |

PL: Panicle Length; DW: Dry Weight of 1000 Seeds; SP: Seeds per Panicle; SL: Seed Length; SW: Seed Width; L/W: Length/Width Ratio; ED: Euclidean Distance

variations. Freire and colleagues [13] affirmed that the culture medium with high concentrations of growth regulators and the prolonged subcultures threaten the genetic fidelity of regenerating plants, since they can origin anomalies in the chromosomes that lead to the production of off-type plants. In addition, the regeneration of Cuban rice cultivar Jucarito-104 occurs through somatic embryogenesis [3] and it has been reported that this process adds specific risks for the appearance of off-type plants, including the unicellular origin of somatic embryos and the use of strong auxins [13].

Some studies have been carried out using molecular markers that have revealed aspects related to genetic and epigenetic instability derived from *in vitro* cultivation of rice. In this sense, Machczynska, et al. [14] found that DNA methylation in regenerated plants doubled its values compared to control plants. Regenerated plants have frequently shown phenotypic alterations, and somaclonal variation has been described in various characters of agronomic interest such as germination rate, growth period, plant height and number of grains per panicle [15], the latter coinciding with the results of this work. Other authors observed the occurrence of somaclonal variation between regenerants in terms of leaf morphology, plant height and number of seeds, with a significant effect of 2.4-D on the mitotic index and the ploidy level of cell nuclei [16].

Regarding the incidence of transgenesis in somaclonal variation, Stroud, et al. [17] carried out a methylation mapping of the rice genome in several regenerated lines transformed with different transgenes and in lines regenerated from non-transformed calli. They detected that all the regenerated plants, transgenic and non-transgenic, showed a significant loss in methylation levels compared to plants from a control population not cultivated *in vitro*, which agrees with the data provided by the present work.

The agronomic importance of genetically modified plants depends fundamentally on the adequate expression of the introduced trait and on the maintenance of the productive, morphological and physiological characteristics of the donor genotype. Improvement of *in vitro* culture conditions, to achieve a plant regeneration protocol with high genetic fidelity, is crucial in the development of an effective program for the improvement of plants through genetic engineering, where it is a practice to ignore transgenic genotypes with undesirable phenotypic traits. In the specific case of the Cuban rice variety J-104, the optimization of 2.4-D concentration and the *in vitro* culture time of the plant material could be an essential point to establish a protocol for the regeneration of plants with high fidelity to the donor genotype. The effectiveness of this protocol can be tested with the Euclidean distance, as a useful practical tool.

## Conclusion

The Euclidean distance algorithm is an easy way to provide new evidences that tissue culture can result in inheritable difference in rice plants (cv. Jucarito-104) with respect to the traditional cultivar. The application of this algorithm also contributes to establishing a more integrated evaluation of the transgenic plants, which is important to validate the phenotypic fidelity of them to the traditional cultivar. However, the study does not detect the genetic changes caused by tissue culture-induced somaclonal variation, which could be demonstrated in future studies based on genomic analysis. This tool would further allow the selection of individual transgenic plants with the least genomic deviation from the traditional cultivar plants.

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**DOI: 10.36959/973/437**