



EVALUATION OF ASGR p779/p780 PRIMERS IN THE DETERMINATION OF APOSPORIC APOMIXIS IN *CENCHRUS PURPUREUS*

EVALUACIÓN DE CEBADORES ASGR p779/p780 EN LA DETERMINACIÓN DE LA APOMIXIS APOSPÓRICA EN *CENCHRUS PURPUREUS*

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The objective of this study was to evaluate the molecular markers ASGR p779/p780, specific for aposporic apomixis, in the determination of the type of reproduction in accessions of *Cenchrus purpureus*. The study was conducted at the Alliance of Bioversity International and the International Center for Tropical Agriculture. The samples under study come from 62 accessions of *C. purpureus* from the Germplasm Bank of Grasses and Forages of Instituto de Ciencia Animal. The type of reproduction of the accessions was determined by Polymerase Chain Reaction (PCR) with the combination of direct and reverse primers p779/p780 completely linked to the aposporic apomixis. It was not obtained DNA amplification from the *C. purpureus* accessions and the *Urochloa* spp. controls that were negative for aposporic apomixis. However, DNA amplification was obtained from positive controls to aposporic apomixis in *Urochloa* spp. accessions reported as apomictic. The amplification products revealed a well-defined polymorphic band on the gels with an approximate size of 950 base pairs. The p779/p780 primers amplified the apomixis-positive *Urochloa* accessions, but did not amplify the negative controls or the *C. purpureus* accessions. These results suggest that (I) or the 62 accessions of *C. purpureus* from ICA reproduce sexually and do not exhibit aposporic apomixis, or that (II) the primers are not specific to this species.

El objetivo del presente trabajo fue evaluar los marcadores moleculares ASGR p779/p780, específicos para apomixis apospórica, en la determinación del tipo de reproducción en accesiones de *Cenchrus purpureus*. El estudio se desarrolló en la Alianza de Bioversity International y el Centro Internacional de Agricultura Tropical. Las muestras en estudio provienen de 62 accesiones de *C. purpureus* del Banco de germoplasma de pastos y forrajes del Instituto de Ciencia Animal. Se determinó el tipo de reproducción de las accesiones mediante Reacción en Cadena de la Polimerasa (PCR) con la combinación de cebadores directo e inverso p779/p780 completamente ligados a la apomixis apospórica. No se obtuvo amplificación del ADN de las accesiones de *C. purpureus* y los controles *Urochloa* spp. negativos a la apomixis apospórica. Sin embargo, se obtuvo amplificación del ADN de los controles positivos a la apomixis apospórica en las accesiones de *Urochloa* spp. reportadas como apomicticas. Los productos de amplificación revelaron una banda polimórfica nítida en los geles con un tamaño aproximado de 950 pares de bases. Los cebadores p779/p780 amplificaron las accesiones de *Urochloa* positivas a la apomixis, pero no amplificaron los controles negativos ni las accesiones de *C. purpureus*. Estos resultados sugieren que (I) o las 62 accesiones de *C. purpureus* del ICA se reproducen de forma sexual y no presentan apomixis apospórica, o que (II) los cebadores no son específicos para esta especie.

Key words: DNA, molecular marker, PCR, reproduction, *Urochloa*

Palabras clave: ADN, marcador molecular, PCR, reproducción, *Urochloa*

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Introduction

The species *Cenchrus purpureus* (Schumach) Morrone is native to Tropical Africa. It is a perennial or rhizomatous geophyte and grows mainly in the seasonally dry tropical biome. It is used as animal food and in medicine; it has environmental and social uses, and is used as fuel (POWO 2025). In *C. purpureus* there are two types of reproduction: sexual (by seeds) and asexual (by vegetative cuttings) (Wessapak et al. 2023).

Apomixis is the phenomenon of clonal reproduction by seed and occurs naturally in more than 400 plant species (Xu et al. 2022). In the genus *Cenchrus*, it is estimated that at least eight species exhibit apomixis, although the numerical accuracy varies due to the biological complexity of the phenomenon and the presence of facultative reproductive modes (combination of apomixis and sexuality) in several species (Kumar et al. 2019).

The identification studies of apomixis in *C. purpureus* were carried out by Hanna (1981) and González and Hanna (1984) for which they used morphological markers and determined in all cases the form of sexual reproduction in accessions of *C. purpureus* and hybrids of *C. purpureus* x *Cenchrus americanus* (L.) Morrone. However, genetic information on the presence of genomic regions involved in this mode of reproduction has not been described in *C. purpureus*.

Among the molecular markers linked to the mode of reproduction in apomictic species in Poaceae, there is the ASGR (apospory-specific genomic region) p779/p780 specific for aposporic apomixis. Developed by Akiyama et al. (2011), from sequences of exons four and seven of the ASGR-BBM-like2 gene of *Pennisetum squamulatum* (L.) syn. *Cenchrus squamulatus* (Fresen.) Morrone and which amplifies a region that includes three introns of 950, 266 and 154 base pairs (bp).

The ASGR p779/p780 primers were used to determine the mode of reproduction of accessions of *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs and four species of *Urochloa* (*Urochloa brizantha* (A. Rich.) R. D. Webster, *Urochloa decumbens* (Stapf) R. D. Webster, *Urochloa humidicola* (Rendle) Morrone & Zuloaga and *Urochloa ruziziensis* (R. Germ. & C. M. Evrard) Crins) from the Genetic Resources Program of the Alliance of Bioversity International and the International Center for

Tropical Agriculture (Alliance), in Colombia (Worthington et al. 2016 and Worthington et al. 2019).

There are few studies in the available literature that use p779/p780 primers in *C. purpureus*. With the aim of providing information on the genetic and reproductive characteristics that contribute to the genetic improvement programs of *C. purpureus*, this study aimed to evaluate the molecular markers ASGR p779/p780, specific for aposporic apomixis, in the determination of the type of reproduction in accessions of *C. purpureus* from the collection of Instituto de Ciencia Animal (ICA).

Materials and Methods

This research was conducted in the DNA Laboratory, belonging to Future Seeds building, at the Alliance of Bioversity International and the International Center for Tropical Agriculture (Alliance), Cali, Valle del Cauca, Colombia.

Plant material: The samples under study were obtained from 62 accessions of *C. purpureus*, with similar regrowth age and cultivation conditions, conserved in the germplasm bank of grasses and forages, belonging to Miguel Sistachs Naya Grasses and Forage Experimental Center of Instituto de Ciencia Animal, San José de las Lajas, Mayabeque, Cuba, located at 22° 53 N and 82° 02 W at 80 m.o.s.l.

DNA extraction and amplification: The modified MATAB method (Risterucci et al. 2000) was used for genomic DNA extraction. The DNA amplification was performed by polymerase chain reaction (PCR), with the combination of direct and reverse primers p779/p780 described by Worthington et al. (2016) (table 1).

The PCR mixture was made in a final volume of 12 µL, using 4 µL of 2X Promega buffer (GoTaq® Green Master Mix), with a final concentration of 0.66X, 0.12 µL of p779 and p780, with a final concentration of 0.2 µM in each primer and 6.76 µL of ultrapure water (UltraPure™ DNase/RNase-Free Distilled Water, Catalog number: 10977015-Invitrogen) and 1 µL of genomic DNA with a concentration of 10 ng. As controls, DNA samples from *Urochloa* accessions, with apomictic and sexual reproduction, from the Genetic Resources Program of the Alliance of Bioversity International and the International Center for Tropical Agriculture, Colombia, were used (table 2).

Table 1. List of primers and their sequences used in the study

Primer	Location	Sequence 5'----3'	Source
p779	Direct	5'TATGTCACGACAAGAATATG'3	(Worthington et al. 2016)
p780	Reverse	3'TGTAACCATAACTCTCAGCT'5	

Table 2. Description of the apomictic and sexual controls used in the study

Species	Accession	Type of reproduction	Control	Name
<i>U. decumbens</i>	CIAT 606	Apomictic	Positive	(C+)
<i>U. ruziziensis</i>	CIAT 6713	Sexual	Negative	(C-)
<i>U. ruziziensis</i>	CIAT 26168	Sexual	Negative	(D-)
<i>U. brizantha</i>	CIAT 16338	Apomictic	Positive	(E+)
<i>U. ruziziensis</i>	CIAT 26295	Sexual	Negative	(F-)
<i>U. brizantha</i>	CIAT 16776	Apomictic	Positive	(G+)
<i>U. brizantha</i>	CIAT 16447	Apomictic	Positive	(H+)

The amplification was performed in an Eppendorf Mastercycler Nexus Gradient Thermal Cyclers Cole-Parmer® USA thermocycler. The PCR reaction was carried out following a program of approximately 2 hours duration. The thermal profile consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles consisting of: denaturation at 94°C for 30 seconds, hybridization at 52°C for 30 seconds and extension at 72°C for 60 seconds. Finally, a final extension was performed at 72°C for 10 minutes to complete the synthesis of the amplified fragments.

Separation of PCR products: The amplified products were analyzed by electrophoresis on a 1.5% agarose gel prepared with GelRed™ (Biotium) as an intercalating agent. The run was performed in 0.5X TBE buffer at 100 V for approximately 2 hours. The 1Kb DNA Ladder molecular weight marker, INVITROGEN®, was used.

Visualization of PCR products: Visualization and analysis of the amplified DNA fragments was performed using photography, with the BIO-RAD ChemiDoc MP Imaging System Universal Hood III, USA photodocumenter.

Results and Discussion

The results showed that the DNA from the 62 accessions of *C. purpureus* and the negative controls to aposporic apomixes of *U. ruziziensis* did not amplify with the ASGR p779/p780 primers. However, amplification of DNA of the positive controls to the apomixes in *U. decumbens* and *U. brizantha* accessions was obtained. The amplification products showed a well-defined polymorphic band on the gels for the apomictic accessions. The amplified fragments showed an approximate size of 950 pb (figure 1).

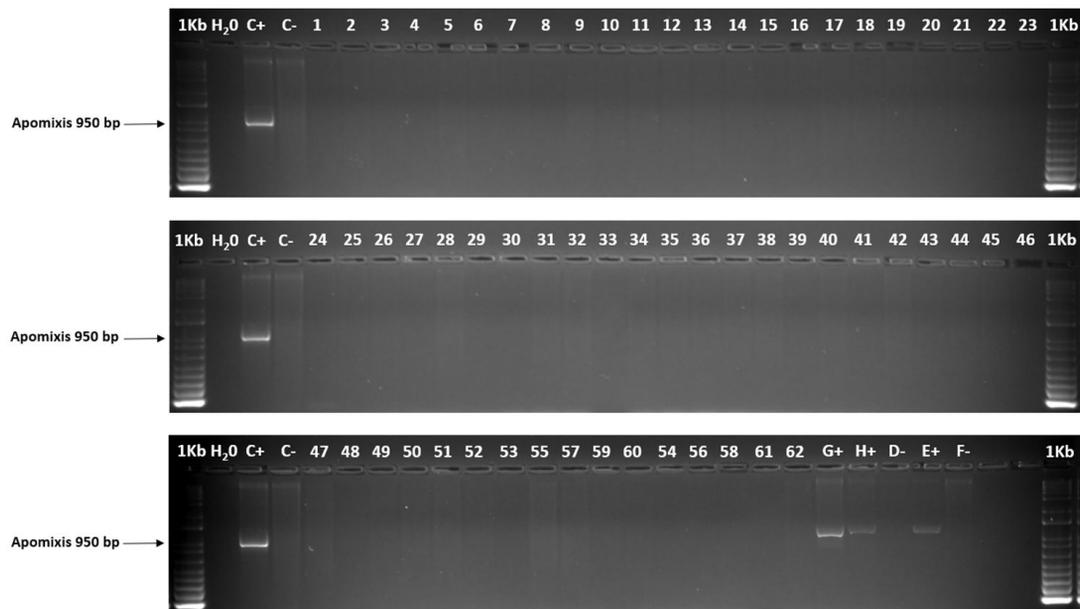


Figure 1. PCR products with the amplification of *Urochloa* spp. control positives to aposporic apomixis and the 62 *C. purpureus* samples without amplification with the ASGR p779/p780 primers. Controls *U. decumbens* (C+), *U. ruziziensis* (C-) (D-) (F-), *U. brizantha* (E+) (G+) (H+).

According to Cook *et al.* (2020) and Genesys PGR (2025) platform, the *U. ruziziensis* accessions 6713, 26168 and 26295 used as negative controls in this study are sexual reproduction materials. However, the accessions *U. decumbens* CIAT 606, *U. brizantha*, 16338, 16776 and 16447 are of apomictic reproduction. This is logical with the results obtained in the PCR. The *Urochloa* spp controls are important indicators in this study since they show the presence or absence of the aposporic and their diversity in species from the same genus.

The PCR results showed that for the samples of *C. purpureus* species there was not amplification in any of the cases. These suggest that the 62 *C. purpureus* accessions preserve in the ICA collection are not reproduce by aposporic apomixis; or these primers are not specific for this species since there was not positive controls for *Cenchrus* genus. Although the information about the use of ASGR p779/p780 primers in *C. purpureus* is limited can cause uncertainty on if they are compatible with the DNA pattern. However, the results of this study, could be confirm the way of sexual reproduction in *C. purpureus* defined by Hanna (1981) and González and Hanna (1984) for which they used morphological markers and determined the reproductive characteristics of the *C. purpureus* accessions and hybrids of *C. purpureus* x *C. americanus*.

The results found in this study in the 62 *C. purpureus* accessions support the Akiyama *et al.* (2011) results, which showed that the specific ASGR p779/p780 primers has a perfect link with the genomic region ASGR in others *Cenchrus* species and the *Urochloa* and *Megathyrsus* genus, where it was confirm their excellent diagnostic capacity for the apomictic reproductive way. Also, these primers support the hypothesis of a common origin for the aposporic apomixis in the Paniceae tribe (Akiyama *et al.* 2011, Worthington *et al.* 2016 and Worthington *et al.* 2019).

On the other hand, the results showed that the ASGR p779/p780 primers consistency amplified the characteristic fragment of approximately 950 pb in the apomictic controls of *Urochloa*, while they do not generate amplification in the sexual controls either in the 62 *C. purpureus* accessions evaluated. This response was coherent with the reproductive performance known from the used controls and confirms the functional of the implemented PCR system (Worthington *et al.* 2016 and Worthington *et al.* 2019).

The absence of amplification in *C. purpureus* can interpreted based on two main hypotheses. The first, and most provable with basis in the available literature, is that the evaluated accessions have a sexual reproduction way. Classic studies performed by morphologic markers has been reported sexuality in *C. purpureus* and in hybrids with *C. americanus* (Hanna 1981 and González and Hanna 1984), which coincides with the lack of detection of the marker associated to

apomixis in this study. Furthermore, comparative studies in the *Cenchrus* genus show that not all species possess the ASGR region or express apomixis, and that sexuality is common within the group (Akiyama *et al.* 2011 and Santos *et al.* 2025).

The second hypothesis considers the possibility that the p779/p780 primers are not fully compatible with the genetic basis of *C. purpureus*. Researchers in apomictic species of *Cenchrus* genus has shown that the ASGR region can undergo chromosomal rearrangements or be located in different positions depending on the species (Goel *et al.* 2006), which could affect the conservation of target sequences for amplification. Additionally, although the p779/p780 marker has shown high diagnostic accuracy in species of *Urochloa*, *Megathyrsus* and some species of *Cenchrus*, it has also been documented that its association is not perfect in segregating populations (da Costa Lima 2023 and Santos *et al.* 2025), so its performance may vary between taxa.

In this regard, although the results support the interpretation of sexual reproduction in the evaluated accessions, it is recommended to include apomictic controls belonging to *Cenchrus* genus in future studies to strengthen the interspecific validation of the marker and rule out specific sequence differences. Likewise, the integration of cytoembryological analyses or genomic approaches would allow confirmation of the presence or absence of the ASGR-BBML region and further characterization of the reproduction mode of *C. purpureus*.

Conclusions

The ASGR p779/p780 primers showed a clear and specific amplification in the apomictic accessions of *Urochloa* spp. there was not amplification in the sexual controls or in the 62 *C. purpureus* accessions evaluated. These results, together with the previous evidence available for the species, suggest that the analyzed accessions of *C. purpureus* reproduce sexually and do not exhibit aposporic apomixis.

However, due to the lack of apomictic controls of *Cenchrus* genus, it is recommended to validate these results using apomictic materials of the same genus, as well as to complement this analysis with cytoembryological or genomic methods that allow for more precise confirmation of the reproduction mode in *C. purpureus*.

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