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COMPARATIVE ANALYSIS OF GENOMIC DNA EXTRACTION METHODS IN LIMA BEAN (*Phaseolus lunatus* L.)

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INTRODUCTION: The lima bean (*Phaseolus lunatus* L.) is the second most important legume of the genus *Phaseolus* (Maquet et al. 1999). In Brazil, this species is mainly cultivated in the Northeast region and presenting great socioeconomic importance for family communities. In that region there are water scarcity and predominate, long periods of drought, what favors the selection of drought tolerant genotypes and adaptation to different environments? The development of research on lima bean Genetics, Genetic Resources and Plant Breeding is extremely important considering its socioeconomic potential and the lack of information for its exploitation. Thus, we aimed to compare five genomic DNA extraction methods and to determine the most efficient protocol for *Phaseolus lunatus*, with a view to genotyping.

MATERIAL AND METHODS: Five protocols of DNA extraction in lima bean were evaluated: Dellaporta et al. (1983); Doyle and Doyle (1987), modified; Khanuja et al. (1999); Ferreira and Grattapaglia (1996), Romano and Brazilian (1999). Quality and integrity of DNA samples of each protocol were observed through the comparative analysis of the intensity and the standard of the bands obtained by agarose (0.8%) gel electrophoresis. The spectrophotometer NanoDrop 2000™ also quantified the samples, which provides the absorbance ratios 260/280 nm in addition to the quantification of the nucleic acids. The gels were stained with GelRedTM and visualized with a photo-documenter. The accessions used to compare the protocols were UFPI 1007 and UFPI 804, from the Germoplasma Bank of the Federal University of Piauí.

RESULTS AND DISCUSSION: The analysis of agarose gel electrophoresis showed that all protocols, except of Khanuja et al. (1999), were efficient in obtaining good amount of high molecular weight genomic DNA of the two lima bean accessions (Figure 1). It also observed little degradation of extracted DNA in all protocols. According to the data obtained in NanoDrop 2000TM (Table 1), the protocol that showed superior with respect to the concentration of DNA was Doyle and Doyle (1987), modified (above 2000 ng / l of DNA), for the two lima bean accessions. The protocols Dellaporta et al. (1983), Doyle and Doyle (1987), Romano and Brasileiro (1999) presented an absorbance ratio 260/280 nm equal to or greater than 1.8. This indicates that the DNA extracted by these protocols is free from contaminants such as phenols and proteins. The DNA of the lima bean accessions, UFPI 1007 and UFPI 804, extracted by the protocols Dellapporta et al. (1983), Doyle and Doyle (1987), Romano and Brasileiro (1999) presented high integrity and quality, suggesting the feasibility of using these protocols.

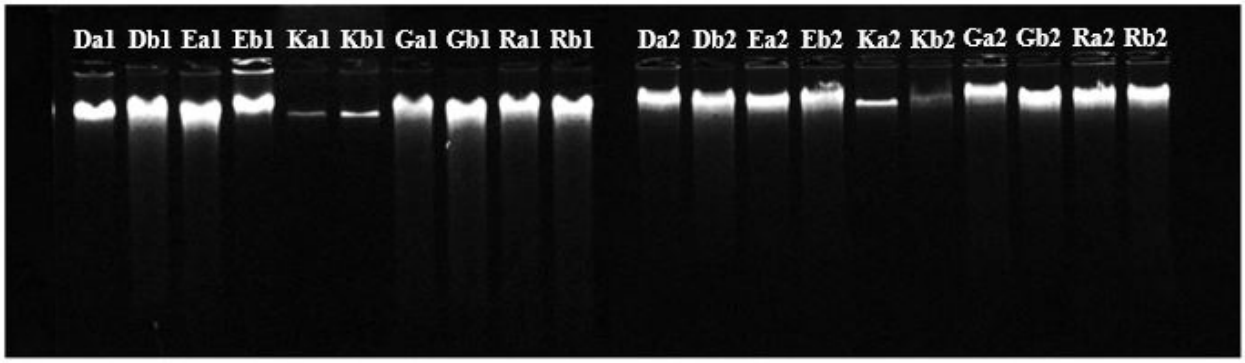

Figure 1. Electrophoretic profile of DNA extracted from young leaves of *Phaseolus lunatus* L. by the methods: D - Dellaporta et al. (1983); E –Doyle and Doyle (1987); K - Khanuja et al. (1999); G – Ferreira and Grattapaglia (1998) and R – Roman and Brasileiro (1998). a - UFPI 1007 and b - UFPI 804. 1 - plant 1 and 2 - plant 2.

Table 1. DNA quantification of two lima bean accessions with spectrophotometer NanoDrop™ 2000.

Amostra*	Concentração ng/µl	260/280	Amostra	Concentração ng/µl	260/280
Da1	1281,3	1,89	Da2	1390,8	1,84
Db1	1084,4	1,72	Db2	1286,3	1,85
Ea1	2712,4	1,89	Ea2	3071,3	1,86
Eb1	2474,5	1,84	Eb2	2009,9	1,93
Ka1	211,6	1,3	Ka2	543,3	1,60
Kb1	344,2	1,52	Kb2	569,9	1,66
Ga1	1231,7	1,82	Ga2	1423,2	1,78
Gb1	1035,7	1,76	Gb2	917,6	1,74
Ra1	1325,2	1,8	Ra2	1624,9	1,85
Rb1	1335,2	1,84	Rb2	1414,2	1,83

*D–Dellaporta et al. (1983); E–Doyle and Doyle (1987); K-Khanuja et al. (1999); G–Ferreira and Grattapaglia (1998) and R-Romano and Brasileiro (1998). a - UFPI 1007 and b - UFPI 804. 1 - plant 1 and 2 - plant 2.

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