Mapping biodiversity of Horse gram (*Dolichos biflorus*) in Eastern India at the seed storage protein level



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Introduction

Horse gram (*Dolichos biflorus* L.) is an important pulse crop in India that is valued for its nutritional and medicinal properties (Fig. 1). Additionally, horse gram is rich in lectins (DBA) which specifically bind N-acetyl-D-galactosamine residues and therefore are widely used as markers i. e. for neuronal pathways and blood type identification. *D. biflorus* lectins belong to a multigene family with a tissue-specific expression pattern.

In this study we focussed on the lectin DBL which is expressed in the seeds (Fig. 2). The tetrameric glycoprotein with a molecular mass of approximately 110 kDa consists of 2 types of subunits. Subunit II (241 aa) is post-translationally processed from subunit I (253 aa).

This ongoing study aims on the identification of structural variation in the polypeptide sequence of DBL.

Materials and Methods

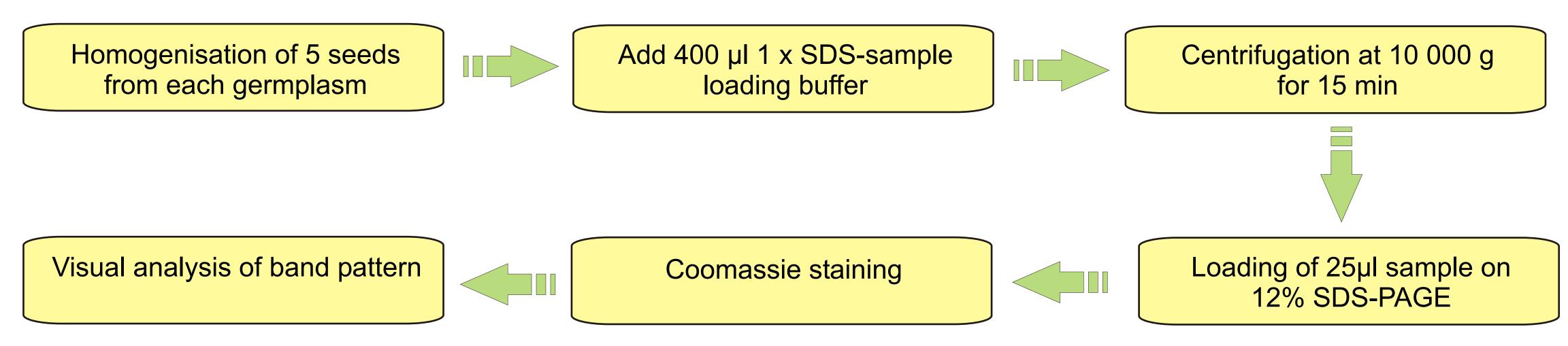
We collected 40 different horse gram germplasms from ethnic farming communities of the Eastern Indian Plateau region in the state Jharkhand (Fig. 3). The generally tropical climate shows regional variations, some areas being heavily draught affected in contrast to heavy long time rainfalls between July and September. We therefore presume that these germplasms show adaptation towards cumulative stress from insufficient water and other associated environmental factors.



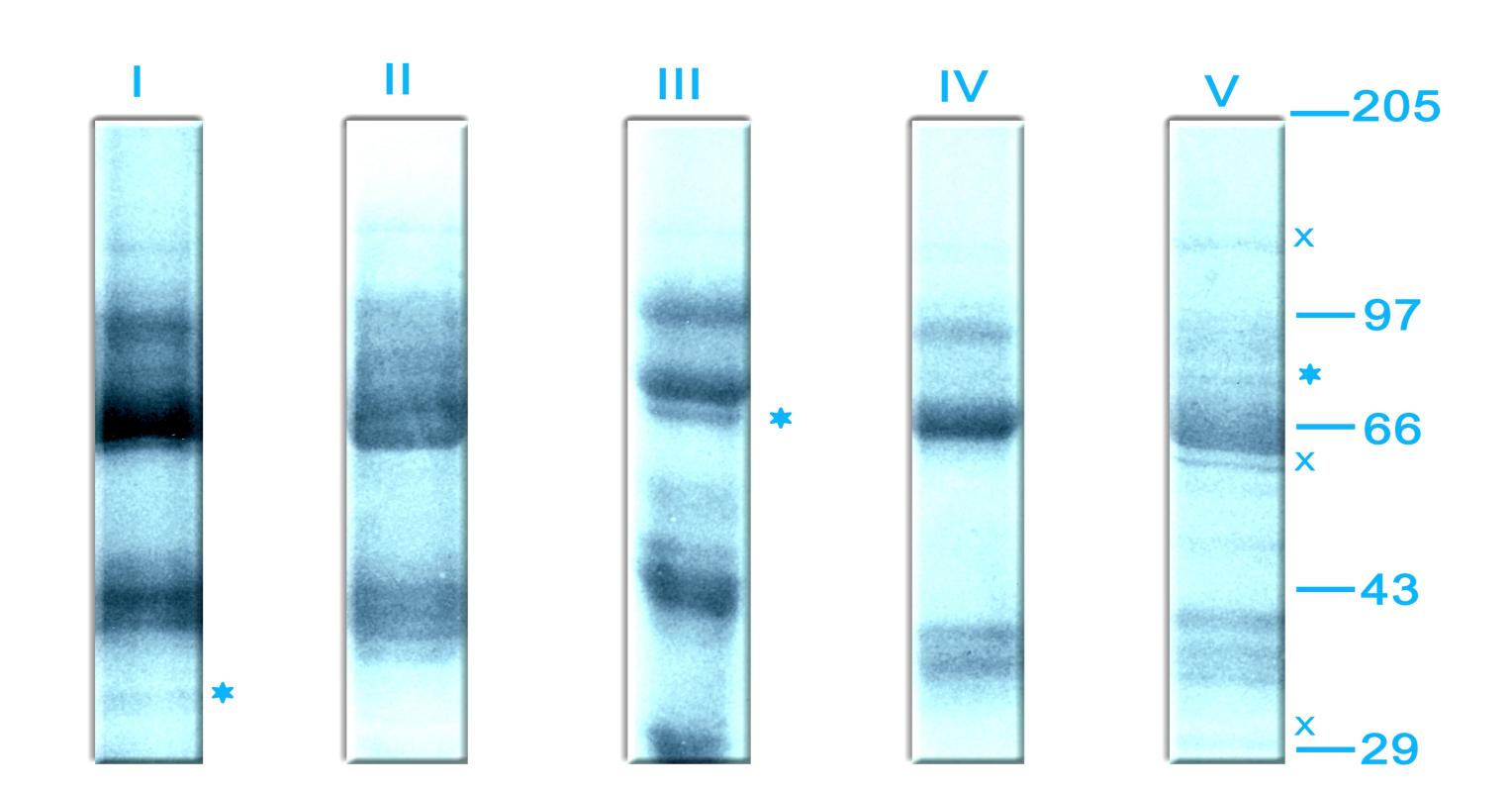
Fig.1 Horse gram plant (Dolichos biflorus L.)

Fig. 2 Seeds of the different horse gram germplasms collected for the analysis.

Fig. 3 The state Jharkhand where samples were collected is marked in the map with dark colour.



Results and Discussion



<u>Fig. 4</u> 40 horse gram germplasms available from the field could be characterized into five distinct groups distinguished by protein band patterns. `I'-`V' represents five broad groups of germplasms identified at the SDS-PAGE level. Numbers on the right hand side represents standard molecular masses in kilodaltons (Kd). `X' represents 30 Kd, 60 Kd and 120 Kd bands, while asterisks represents unique bands in some of these groups of germplasms.

The gel patterns showed that 40 germplasms could be clustered into five groups. Figure 4 shows representatives of each of these five classes. Table 1 describes the exact distribution of these bands in these five groups. As the approximate molecular weight of the DBL monomer is 30 kd we focussed on that area of the gel. We found four closely spaced bands: 32 Kd, 31 Kd, 30 Kd, 29 Kd. The 29 Kd band is present in all the groups, whereas other three bands present in subsets of these five groups. Additionally we found other bands unique to some classes.

Table 1. Characterization of 40 different germplasms of horse gram at the SDS-PAGE level. Distribution of protein bands of germplasms in five different groups:- I^a -12 germplasms; II^b- 9 germplasms; III^c- 4 germplasms; IV^d- 11 germplasms; V^e- 4 germplasms. `+': band observed; `-': band not found.

Approx. Molecular weight of protein bands (Kd)	Group I ^a	Group II ^b	Group III ^c	Group IV ^d	Group V ^e	Band unique to group (s)
130	+	-	-	-	-	I
125	+	+	-	-	+	I, II, V
120	+	+	+	+	+	All
115	-	-	-	+	-	IV
100	+	-	-	+	+	I, IV, V
95	+	-	+	+	-	I, III, IV
85	-	+	-	-	+	II, V
80	+	+	-	+	+	I, II, IV, V
66	+	+	+	+	+	All
64	+	+	+	-	+	I, II, III,V
60	-	-	+	-	+	III,V
50	-	-	+	-	+	III,V
40	-	-	+	-	+	III,V
38	+	+	+	+	+	All
36	+	+	+	+	+	All
34	-	+	-	-	+	II,V
32	+	+	-	-	-	I, II
31	+	+	-	-	-	I, II
30	-	+	+	-	-	II, III
29	+	+	+	+	+	All

Conclusion

From this work we can conclude that horse gram cultivars of different origin show distinct variation in protein composition. Currently we investigate whether this variation concerns DBL, the horse gram seed lectin, as this would raise great opportunities for further research and application in biotechnology.