

Research paper

Character association and molecular characterization of winged bean (*Psophocarpus tetragonolobus* L.)

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ABSTRACT

In order to select high yielding winged bean genotypes, genetic variations between the winged bean genotypes were studied to determine the genetic link between seed yield and yield contributing factors. A study was conducted during *khariif* season 2021-2022 on morphological and molecular characterization of 20-winged bean genotypes (*Psophocarpus tetragonolobus*) for 10 quantitative traits along with 16 ISSR markers were used to evaluate promising and suitable genotypes for cultivation and research. According to the findings of the study, it was observed that the genotype 'IC 112416' exhibited the greatest pod length, measuring 17.8 cm, as well as the highest number of seeds per pod with an average value of 11.8 seed/pod. We found similar results from the study of genetic components and association analysis as PCV (24.4), GCV (23.25), heritability (90.15%) and genetic advance (45.48%). Here we have standardized a DNA extraction protocol for isolating a quantified DNA using CTAB and PVP extraction method, which is further used in different molecular studies. From the molecular analysis it was found that 12 markers were polymorphic in nature with high allelic frequency. The PIC (Polymorphic information content) of each marker is recorded and cluster analysis of molecular data is done using Jaccard's similarity coefficient with the help of dendrogram.

Key words: Association, DNA extraction, Genetic components, ISSR, Winged bean

INTRODUCTION

The winged bean (*Psophocarpus tetragonolobus*) chromosome number $2n=18$, also known as cigarillos, goa bean, four-angled bean, four-cornered bean, manila bean, princess bean, asparagus bean, dragon bean, is a tropical autogamous legume crop with different usage (Mohanty *et al.*, 2013). It has originated from New Guinea. It can be grown as a grain legume, green vegetable, tuber crop, forage and a cover crop (Lepcha *et al.*, 2017). It thrives in the hot and humid climates of South and Southeast Asia. It is an underutilized plant with the potential to become a significant multi-purpose food crop in Asia, Africa, and Latin America's tropics. (Lepcha *et al.*, 2017). The winged bean species are the perennial herbs belongs to the genus *Psophocarpus* which is part of the legume family. *Psophocarpus* species have tuberous roots and pods with wings. They need mechanical support for climbing (Wong *et al.*, 2000).

Winged bean is a good source of protein mainly for the tropical regions and is popular as a protein rich legume crop which promotes abundant

nodulation and high nitrogen fixation (Singh *et al.*, 2016). These properties make the plant withstand extreme conditions. Moreover, they are suitable for cultivation in soils with low fertility and used as a cover crop (Wong *et al.*, 2000; Adegboyega *et al.*, 2019). It is generally consumed as green tender pods sliced or chopped which contain abundant minerals and vitamins. Seeds can be used in similar ways as the soybean. The seed protein content ranges from 30-37% and oil content is about 15-18%. Its leaves, pods, stem, seeds, flowers and fleshy roots can be consumed as a raw meal due to which it is known as "One man supermarket" (Lepcha *et al.*, 2017). For these reasons, this unheeded and underutilized crop has received increased attention in recent years for its potential to enhance the agro biodiversity, high nutrition contents and ability to tolerate or resist quite a few biotic and biotic stresses.

The accumulation and assessment of genotypes are an essential part of any breeding program. Lack of genetic diversity trammels the development of improved varieties of crops, by

reducing the availability of useful alleles and gene-combinations. It plays a key role in plant breeding since progeny obtaining from divergent parents exhibit greater heterosis and provide wide spectrum of variability in the segregating generations. The rapid development of molecular markers has provided new tools for more rapid and precise evaluation of the genetic diversity. In this study, we established a standardized technique for the extraction of DNA from winged bean leaf samples. Additionally, a molecular characterisation of winged bean genotypes was conducted based on the extracted DNA. The hypothesis posits that there is considerable variation in molecular features among different genotypes of winged beans, which might potentially be utilized in a breeding effort.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of the Raj Mohini Devi College of Agriculture and Research Station, Ambikapur (Chhattisgarh) during the *kharif* season 2021-22 in Randomized block design where the test material and the checks were randomly replicated in several blocks. The experiment consisted of a total of 17 genotypes and 3 control groups. They were randomly assigned and reproduced in 3 rows, each including 20 blocks. The genotypes referred to as RWB-32, RWB-33, RWB-34, RWB-36, RMDWB-20-1, RMDWB-20-2, RMDWB-20-3, AKWB-1, Indira winged bean-1, RMDWB-1, IC 112416, RWBGP-3, EC 178319, EC 178335, IC 026946, RWB-25, RWB-26, RWB-30, RWB-17-14, and RWB-17-18 are being studied. All the recommended package of practices for the region was followed to raise a good crop. Data of all 10 qualitative characters for all 20 genotypes were recorded.

Morphological evaluation

The characters of winged bean were recorded in different growth stages on the basis of an average of 5 plants from each plot separately of all the genotypes. Five plants were selected randomly on the basis of morphology for taking observation. The characters recorded were 50% days to flowering, days to pod setting, pod length (cm), number of pods per plant, number of seeds per pod, seed weight (g), flowering to fruit setting, days to maturity, seed yield, green pod yield. The characters were recorded and the data was analysed using a different standard statistical procedure (Mean, Range, P.C.V, G.C.V, Heritability, Genetic advance, correlation coefficient factors, path analysis in

different statistical software's namely OPSTAT and STAR 2.0.1. Molecular data were recorded after PCR amplification and visualization using gel documentation unit.

Molecular evaluation

DNA extraction: A quantified quality DNA was isolated from the tender green leaves of 20-winged bean genotypes. DNA was extracted according to the modified CTAB protocol using 1% PVP (polyvinyl polypyrrolidone). DNA concentration was determined using agarose gel electrophoresis, stained with ethidium bromide solution and visualization on UV transilluminator. The isolated DNA was checked for quality and then diluted at a specified concentration and used in different molecular researches.

DNA amplification using ISSR markers: DNA was PCR-amplified by using 16 ISSR markers in a 96-well applied Biosystems 2720 thermal cycler. Reactions were carried out in a total volume of 22 μ l/sample consisting of 2 μ l (20 ng) of template DNA, 2 μ l of primer, 13.5 μ l of molecular biology grade water, 2.5 μ l of PCR buffer, 1.5 μ l of dNTPs (mix) and 0.5 μ l of taq polymerase. Amplification was performed under the following conditions: initially 5 min at 94°C for 1st cycle, followed by 0.5 min at 94°C, 0.5 min at annealing temperature (depending on the primer used), 1 min at 72°C for 35 cycles and lastly 7 min at 72°C for final extension. The amplification products were suspended for electrophoresis on 2% agarose gel in 1X TAE buffer. Visualization of amplifications was done on Gel documentation unit by using E-BOX CX5.TS (20M) and analysed for Polymorphism Information Content (PIC). Cluster analysis of recorded molecular data has been done using un weighted Pair-Group Method with Arithmetic Average (UPGMA) and Jaccard's similarity coefficient by the help of dendrogram.

RESULTS AND DISCUSSION

This study combined the use of molecular markers and quantitative traits to investigate various genotypes of winged beans. The present study was carried out throughout the temporal period of the Kharif season, which extended from 2021 to 2022. The genetic parameters such as genotypic co-efficient of variation, heritability and genetic advance were also estimated to study the extent of genetic variability more precisely. The phenotypic variance was further partitioned into its genotypic and environmental

components to know the genetic variability present in each character. It was observed that for most of the characters, phenotypic component of variance was higher than genotypic component and genotypic component of variance was higher than environmental components, inferring that phenotypic variability was a reliable measure of genotypic variability which indicates selection would be effective for these characters. The highest phenotypic and genotypic variances were recorded for pod length with GCV (23.25) and PCV (24.49). The estimates of heritability (h^2) and genetic advance as percentage of mean was also observed high for the characters pod length (45.48%) and seeds per pod (22.834%). These results conjectured that the traits pod length and number of seeds per pod are linked and are directly contributing in the yield enhancement. In the current study, the character seed yield recorded its highest for the treatment IC 112416. The estimates of the genetic parameters for the characters under study are given in table 1. The graphical comparison of the heritability and genetic advance of the characters under study is given in figure 1 and 2, respectively.

Character association analysis

The analysis of degree of association among the characters helps in determining the important characters that need to be considered during selection. The correlation analysis revealed that both green pod yield and seed yield had a significant positive correlation (all genotypic correlation

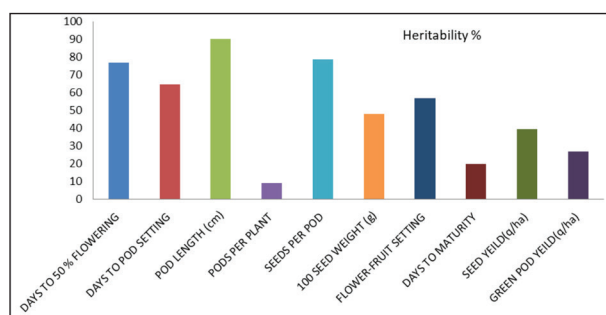


Fig. 1. Heritability of the characters under study

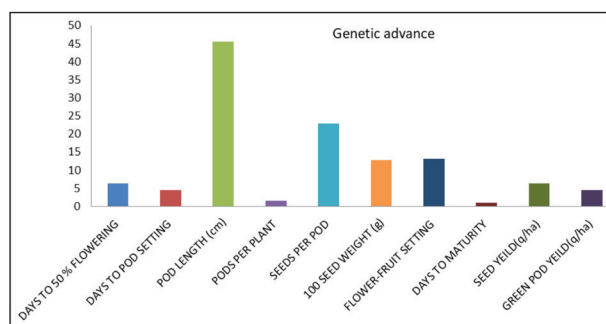


Fig. 2. Genetic advance of the characters under study

coefficient and most phenotypic correlation coefficients) with all of the characters under study, except days to maturity. The correlation of these yields was non-significant with flowering to fruit setting (days). Green pod yield had highest positive correlation with the number of days to pod setting followed by the number of pods per plant and days to 50% flowering whereas days to maturity showed the maximum negative correlation with it. Seed yield also showed the highest positive correlation with the number of days to pod setting followed by the number of seeds per pod. The estimated correlation coefficients are mentioned in table 2.

Table 1. Genetic parameters of variability for yield and attributes in winged bean

Characters	General mean	Range		PCV (%)	GCV (%)	Heritability h^2 (%)	Genetic advance	GA as % of mean
		Min	Max					
DF	69.86	65.66	74.66	3.98	3.49	77.04	4.41	6.32
DPS	78.01	74.3	82.66	3.33	2.67	64.71	3.46	4.44
PL	13.05	9.53	17.86	24.49	23.25	90.15	5.93	45.48
PPP	15.58	13.53	17.10	7.79	2.38	9.34	1.23	1.50
SPP	10.23	8.00	11.86	14.09	12.49	78.65	2.33	22.83
SW	32.03	25.06	37.16	12.85	8.91	48.06	4.07	12.73
FFS	8.46	7.00	9.66	11.16	8.41	56.87	1.10	13.07
DM	156.73	153.33	161.66	1.82	0.81	19.92	1.17	1.05
SY	12.35	10.66	13.73	7.83	4.93	39.69	0.79	6.40
GPY	120.48	107.26	133.8	8.19	4.24	26.85	5.45	4.53

Here, DF-Days to 50% flowering (days), DPS-Days to pod setting (days), PL-Pod length (cm), PPP-Pods per plant (no.), SPP-Seed per pod (no.), SW-Seed weight (g), FFS-Flowering to fruit setting (days), DM-Days to maturity, SY-Seed yield (q/ha) and GPY-Green Pod yield (q/ha).

Table 2. Estimation of genotypic (G) and phenotypic (P) correlation coefficient among various characters in winged bean

		DF	DPS	PL	PP	SPP	SW	FFS	DM	SY
DPS	G	0.934**								
	P	0.852**								
PL	G	0.064	0.037							
	P	0.024	-0.002							
PP	G	-0.164**	-0.107**	1.000**						
	P	-0.084	-0.115	0.263*						
SPP	G	0.524**	0.447**	0.792**	0.928**					
	P	0.379**	0.275*	0.681**	0.415**					
SW	G	0.471**	0.264*	0.824**	1.000**	0.968**				
	P	0.235	0.158	0.579**	0.155	0.669**				
FFS	G	-0.620**	-0.423**	-0.197	-0.206	-0.761**	-0.805**			
	P	-0.424**	-0.289*	-0.093	-0.167	-0.416**	-0.403**			
DM	G	-0.121	-0.236	-0.303*	-0.544**	-0.419**	-0.065	0.025		
	P	-0.135	-0.147	-0.133	-0.167	-0.200	0.172	0.044		
SY	G	0.502**	0.663**	0.455**	0.554**	0.588**	0.399**	-0.168	-0.104	
	P	0.238	0.155	0.317*	0.184	0.357**	0.179	-0.083	-0.364**	
GPY	G	0.764**	0.935**	0.380**	0.924**	0.678**	0.296*	-0.053	-0.873**	0.802**
	p	0.365**	0.409**	0.243	0.112	0.317*	0.134	-0.040	-0.236	0.395**

**Significant at 1%; Significant at 5%; Here, DF-Days to 50% flowering (days), DPS-Days to pod setting (days), PL-Pod length (cm), PPP-Pods per plant (no.), SPP-Seed per pod (no.), SW-Seed weight (g), FFS-Flowering to fruit setting (days), DM-Days to maturity, SY-Seed yield (q/ha) and GPY-Green Pod yield (q/ha).

The coefficients generated by path analysis measure the direct and indirect influence of one variable upon another. From the results of path revealed that the number of seeds per pod had the highest direct positive effect on the seed yield followed by pod length. The other traits showing direct significant positive effect with seed yield are days to 50% flowering and green pod yield, and the traits showing direct negative effect are days to flowering, number of pods per plant, seed weight, days from flowering to fruit setting and days to maturity and seed weight showing the highest negative direct effect. The path correlation estimates of the characters are given in table 3. Yadav (2021) conducted a study on coheritability and correlation of various characters in winged bean and reported that seed yield/plant had the highest estimates of significant positive correlation with days to 50% flowering (0.408, 0.344) and days to maturity (0.330, 0.285). In another study on winged bean, Mohamadali and Madalageri (2004) found that both the path and correlation shows that green pod yield per plant had high significant positive association with the number of pods set per plant and green pod yield. The inferences drawn from the character association analysis in the current study is in good agreement with the findings of these previous researchers. Apart from these traits, the current study also found the characters like pod length, number of seeds per pod and days to 50% flowering

are also having quite significant positive influence on the yields of winged bean.

DNA extraction protocol

Winged bean has been quite unexplored by the geneticists and hence proper DNA extraction protocol for the crop has not yet been established. In the current study, the DNA extraction for molecular analysis was also standardized for winged bean. Before starting the process the mortar, pestles and the scissor were cleaned and wiped with 70% ethanol. After this, 20 μ l of β -Mercaptoethanol was added to 20ml of 1XCTAB for the preparation 2XCTAB solution. The leaf sample was cut using into fine pieces into the mortar and crushed using 2XCTAB extraction buffer at an amount of 400 μ l added 3-4 times during the process of crushing. The ground material was collected in a 2ml eppendorf tube and 8mg of PVP was added in each tube. It was then allowed to mix thoroughly and the tubes were incubated in water bath at 65°C. The samples were allowed to be cooled and then 900 μ l of CIA (Chloroform: Iso-amyl alcohol in the ratio 24:1) was added in each tube. After gentle mixing the tubes were again subjected to centrifuge for 15 minutes at an rpm of 13000. After centrifugation, the upper coloured liquid known as supernatant is collected in a fresh 1.5 ml eppendorf tube and the rest material was discarded along with the tube. The supernatant collected is then added with 900 μ l of Absolute

ethanol per tube and the solution was mixed by inverting the tubes, and refrigerated at -20°C for 2 hours. After 2 hours the tubes were at first manually mixed and then centrifuged at an rpm of 3000 for 8 minutes. After centrifugation the heavy DNA settled down at the bottom of the tube known as DNA pellet. The DNA pellet was then washed with 70% ethanol and centrifuged for 3 min at an rpm of 3000 and then air dried for 30 minutes. Then 50 ml of TE buffer was added to the air-dried DNA pellet and shaken in a way such that the pellet breaks into pieces and dissolved. This was the main stock of the DNA. Figure 3 shows the image of the extracted DNA run on agar gel as seen in the gel documentation unit.

Molecular evaluation

The extracted and quantified DNA of the genotypes under study was then used for fingerprinting using ISSR markers. Sixteen ISSR markers have been used for molecular characterization of winged bean, out of which 4 markers were found monomorphic and 12 markers were polymorphic. The PIC values of the primers were ranging from 0.311 (UBC-885) to 0.808 (UBC-842). The allelic frequency and PIC values of the polymorphic primers are given in table 4. The microsatellite markers showing polymorphism were UBC-842 (Fig. 4-A), UBC-809 (Fig. 4-B), UBC-836, UBC-840, UBC-815, UBC-841, UBC-834, UBC-

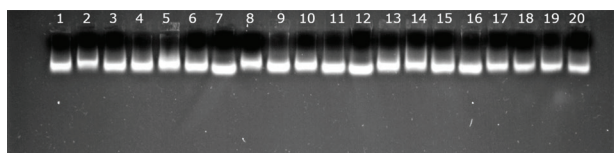


Fig. 3. Image showing DNA of 20 genotypes of winged bean and process

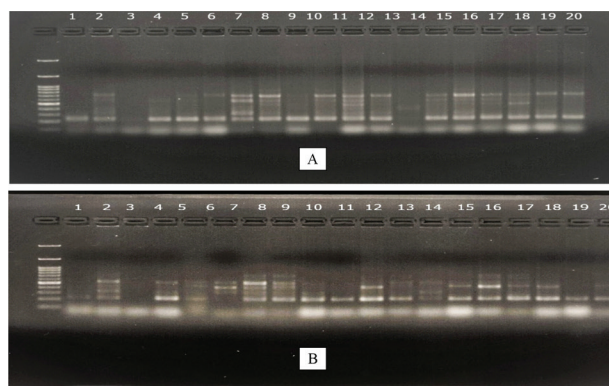


Fig. 4. Amplified PCR product of different DNA using ISSR markers named UBC842 (A) and UBC809 (B).

811, UBC-885, UBC-807, UBC-810 and UBC-884. From the molecular analysis we found the genotypes AKWB-1, Indira winged bean-1 and IC 112416 as more diversified with more polymorphism. Based on the molecular results and cluster analysis we concluded these 3 genotypes as highly suitable for selection for further crop improvement programs. The ISSR markers provided unique information to most of the genotypes. Molecular characterization aids the conventional breeding programs by making the selection procedure more precise and rapid. The molecular markers in the current also generated locus specific allele information, which can be used in future to further generate molecular identity for winged bean genotypes. Besides the yield parameters, molecular markers can also be utilized for development of pest and disease resistant lines. There is a need for precise phenotyping, mapping of QTLs associated with insect resistance, and use them in conjunction with morphological and biochemical markers to develop cultivars with resistance to insect pests (Sharma *et al.*, 2008).

Table 3. Estimation of path coefficient analysis of the following genotypes of winged bean

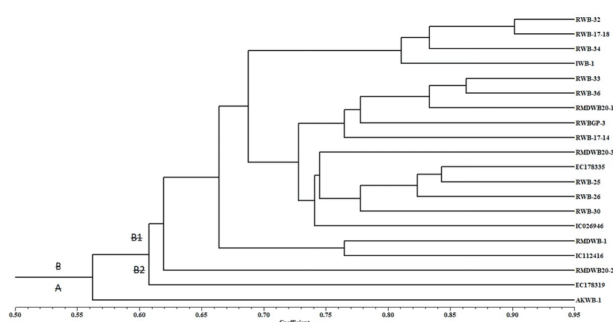
	DF	DPS	PL	PPP	SPP	SW	FFS	DM	SY
DF	0.326	-0.456	0.061	0.090	0.853	-0.842	0.174	0.031	0.262
DPS	0.304	-0.488	0.035	0.058	0.721	-0.471	0.119	0.061	0.321
PL	0.020	-0.018	0.965	-0.582	1.276	-1.472	0.055	0.079	0.130
PPP	-0.053	0.052	1.022	-0.550	1.496	-1.930	0.057	0.142	0.317
SPP	0.172	-0.218	0.763	-0.510	1.613	-1.789	0.214	0.109	0.233
SW	0.153	-0.128	0.795	-0.594	1.615	-1.787	0.226	0.017	0.102
FFS	-0.202	0.206	-0.190	0.113	-1.228	1.438	-0.281	-0.006	-0.018
DM	-0.039	0.115	-0.292	0.299	-0.675	0.116	-0.006	-0.261	-0.300
SY	0.249	-0.456	0.366	-0.508	1.094	-0.529	0.014	0.228	0.344

Residual value = 0.194; Here, DF-Days to 50% flowering (days), DPS-Days to pod setting (days), PL-Pod length (cm), PPP-Pods per plant (no.), SPP-Seed per pod (no.), SW-Seed weight (g), FFS-Flowering to fruit setting (days), DM-Days to maturity, SY-Seed yield (q/ha) and GPY-Green Pod yield (q/ha).

Table 4. Size and frequency of alleles generated by the 12 ISSR markers loci in 20 genotype of winged bean

S. No.	ISSR markers	Marker sequence	Amplification temperature (°C)	Allele number	Major allele frequency	PIC value
1	UBC809	AGAGAGAGAGAGAGAGG	45.1	6	0.435	0.736
2	UBC811	GAGAGAGAGAGAGAGAC	44.8	3	0.791	0.346
3	UBC815	CTCTCTCTCTCTCTCTG	44.7	4	0.4	0.698
4	UBC807	AGAGAGAGAGAGAGAGT	45.2	2	0.681	0.436
5	UBC810	GAGAGAGAGAGAGAGAT	45.1	2	0.666	0.445
6	UBC841	GAGAGAGAGAGAGAGAGC	49.1	4	0.633	0.535
7	UBC840	GAGAGAGAGAGAGAGAAT	45.8	5	0.326	0.772
8	UBC834	AGAGAGAGAGAGAGAGAT	45.5	4	0.347	0.681
9	UBC836	AGAGAGAGAGAGAGAGGA	49.1	6	0.318	0.795
10	UBC885	TATGAGAGAGAGAGAGA	41.7	3	0.818	0.311
11	UBC842	GAGAGAGAGAGAGAGAGG	49.1	7	0.31	0.808
12	UBC884	ATAAGAGAGAGAGAGAG	41.7	2	0.678	0.438

For cluster analysis, the PCR generated markers alleles for each genotype were converted into binary scores based on their presence or absence as '1' and '0', respectively following the Jaccard's similarity coefficient (UPGMA) algorithm computed by NTSYS- pc (Numerical Taxonomy and Multivariate Analysis) software version 2.1 (Rohlf, 2000). The analysis was performed using Jaccard's similarity coefficient matrices calculated from ISSR markers to generate a dendrogram of 20 genotypes. The analysis revealed the genotypes as divided into 2 major clusters at similarity coefficient of 0.56 (Cluster A and B). Cluster A contained only 1 genotype and rest of the lines lied in cluster B. Cluster B was subdivided into cluster B1 and B2 at similarity coefficient of 0.61. Genotypes RWB-32 and RWB-17-18 showed highest similarity percentage of 90% (similarity coefficient 0.90). Figure 5 shows the dendrogram generated by the cluster analysis.

**Fig. 5.** Dendrogram representing different clusters

CONCLUSION

Winged bean genotypes show wide variation for selected traits along with high heritability.

Therefore, it is concluded that the characters which showed high genotypic value coupled with high heritability and genetic advance should be considered for direct selection. Since the trait pod length is recorded high GCV (23.25%), PCV (24.49%), heritability (90.15%) and genetic advance (45.48%) and used for direct selection. From the mean performances, we found that the genotype named IC 112416 was recorded highest for pod length (17.8 cm), and number of seeds per pod as (11.8) and seed yield as (13.7 q/ha). 16 ISSR markers have been used for molecular characterization of winged bean, out of these 4 markers were monomorphic and 12 markers were polymorphic. The microsatellite markers showed more allelic number were UBC-842, UBC-809, UBC-836, UBC-840, UBC-815, UBC-841, UBC-834, UBC-811, UBC-885, UBC-807, UBC-810, UBC-884 and the genotypes showing high frequency of polymorphism were AKWB-1, Indira winged bean -1 and IC 112416.

Authors' contribution

Conceptualization of research (JK); Contribution of experimental materials (JK); Execution of field/lab experiments and data collection (TP, JK, AB); Preparation of manuscript (JK, TP); Analysis of data and interpretation (JK, TP)

DECLARATION

The authors declare no conflict of interest.

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