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GWAS of important crops of Amaranthaceae family with special reference to *Chenopodium*: A review

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Abstract

Wide association of genomes deals with identifying naturally occurring genetic variance with targeted traits or genes. Putative candidate genes had the capability for improvement in quality and resistance to biotic and abiotic stress by exploiting linkage disequilibrium. Plants of the Amaranthaceae family like Spinach, *Amaranthus, Chenopodium* and Sugarbeet are packed with essential nutritional components and are resistant to several biotic and abiotic stress. Several candidate genes are identified for the improvement of floral development, early flowering, late flowering, bolting formation and resistance to several biotic and abiotic stresses . Through GWAS study, the genetic basis of several complex trait phenotypes can be deciphered for important agricultural crop plants. Exploiting these plants through GWAS will allowed knowing the putative candidate genes present in them which could be identified and used for further improvement of the crops.

Keywords

Candidate gene; Linkage disequilibrium; Quantitative Traits Loci; Single Nucleotide Polymorphism

Introduction

The plants of the Amaranthaceae family are rich sources of vitamin E and they have the potential to relieve diseases like cardiovascular and hypertension by lowering cholesterol levels and blood pressure. Studies also showed that many other diseases like anaemia, prostate cancer and osteoporosis can be reduced by consuming plants from the Amaranthaceae family (1). The plants in this family are packed with rich sources of vitamins and minerals (2). Among the minerals content, it is a rich source of potassium which provides health benefits like strengthening respiratory function along with better muscular function and also helps in preventing hypertension. Secondary metabolites like phenolic acid and flavonoids are present in most of the plants under this family (3). Also, the plant has several characteristics like high diversity, resistance to several biotic and abiotic stresses and high adaptability to environment and climate change (4). The plants also have climate-resilient properties which increase the demand for the crop and its cultivation. Besides having high nutrient content, the salinity and drought tolerance properties of the plant make the crop a promising vegetable to acclimatize to the effects of climate change (5). Also due to the C4 mechanism of the plant under this family, it can be grown as a drought-resilient and salinity-tolerant crop by reducing water loss during photosynthesis (6). Amaranth grain is rich in nutrients like proteins and a balanced amino acid along with dietary fibers and minerals like Fe, Ca and Zn.

Due to its absence of gluten, the plants under this family are highly demanded (1, 7,). Despite having climate-resilient and high nutrient content of the plants, less research work is done to understanding the genes responsible for such important traits and still, more research work needs to be performed. Through forward genetics, individuals who differ in genotype are screened back to underlying causative loci to understand the link between phenotype and genotype. One approach for this is Genome-Wide Association Studies (GWAS). Plants under the Amaranthaceae family are packed with important properties and GWAS is one important method to find out the causative loci and candidate genes responsible for those characteristics. While trying to understand the crop more deeply, it is necessary to understand the level of research work done to date to understand the status of research in the crop.

GWAS as important genomic tools

Understanding the genotypic polymorphism has been a biological interest. Several mapping approaches such as quantitative trait loci (QTL) mapping and genome-wide association studies (GWAS) can be used to understand the link between phenotypic and genotypic differences. QTL mapping helped in finding the gene locus that co-segregate with a desired phenotypic trait. QTL is an important molecular tool to understand the loci linked to a trait. Traditional QTL mapping depends on how diverse the two parents are and the effect of detection can differ between populations. Despite having a successful application of QTL mapping in understanding phenotype-genotype interaction, it still faced two main drawbacks. The first is the segregation of only allelic diversity between the parents within the recombinant inbred line (RIL) population or F_2 cross. The second drawback is the limitation in the mapping resolution of the RIL population due to the occurrence of recombination. GWAS overcomes these two main drawbacks of QTL analysis. The regions of candidate genes present in QTL analysis can be quite large. While in GWAS, a large number of markers are being used which can capture all the possible SNPs and thus reduce the number of candidate genes present which can improve the identification of direct genes responsible for the trait of interest (8). The basic approach of GWAS is the evaluation of the association between each marker genotype and phenotype of interest across several individuals. A pictorial representation of the general procedure for Genome-Wide Associated Studies (GWAS) is presented in Fig. 1. GWAS served as a foundation experiment to understand the genetic architecture of the trait which suggests the candidate gene for transgenic and mutagenesis. It also decides the choice of parents for QTL analysis. So, QTL mapping when conducted together with the GWAS study can mitigate each other's limitations (9, 10).

The first successful GWAS was performed to identify a variant associated with age-related molecular degeneration in the Complement Factor H gene (11). GWAS exploits the linkage disequilibrium (LD) of natural populations and identifies naturally occurring genetic variances with targeted traits. The detection of the association between the trait and DNA variants depends on the size of the experimental sample, frequency of genetic variants, distribution of causative genetic variants segregating in the population and LD value observed between the unknown causal variants and observed genotyped DNA variants. Therefore, the success of GWAS for a particular trait depends on the number of loci associated with the trait segregate in the population, the distribution of allele frequency and effect size at those loci. It also depends on the size of the experimental sample and the heterogeneous nature of the trait under study.

A combination of GWAS and QTL analysis can improve the limitations of separate approaches of QTL analysis and GWAS and thus help in identifying the loci controlling the agronomically important quantitative traits (12). Several reports have been found where the combined study of QTL and GWAS analysis has been performed in several crop plants (13). Single-locus GWAS can be performed by following different procedures like mixed linear model (MLM) and general linear model (GLM) where QTLs having only two alleles will be identified in plants (15). While a larger number of QTLs can be identified by using multiallele and multilocus GWAS (RTM-GWAS) to enable the advantage of identification of a large number of QTLs and their allele constitutions (14).

One drawback for GWAS is the presence of false positive error so validation of the result is necessary. One of the sequencings methods or genotyping methods used in GWAS is Genotyping by sequencing and GBS-GWAS. During the GWAS procedure, errors in genotype calling can happen and sometimes also due to low-quality samples, there are high chances of false-positive and false-negative findings. Quality control (QC) is one such procedure that can be used for the removal of low-quality markers or samples. Some QC

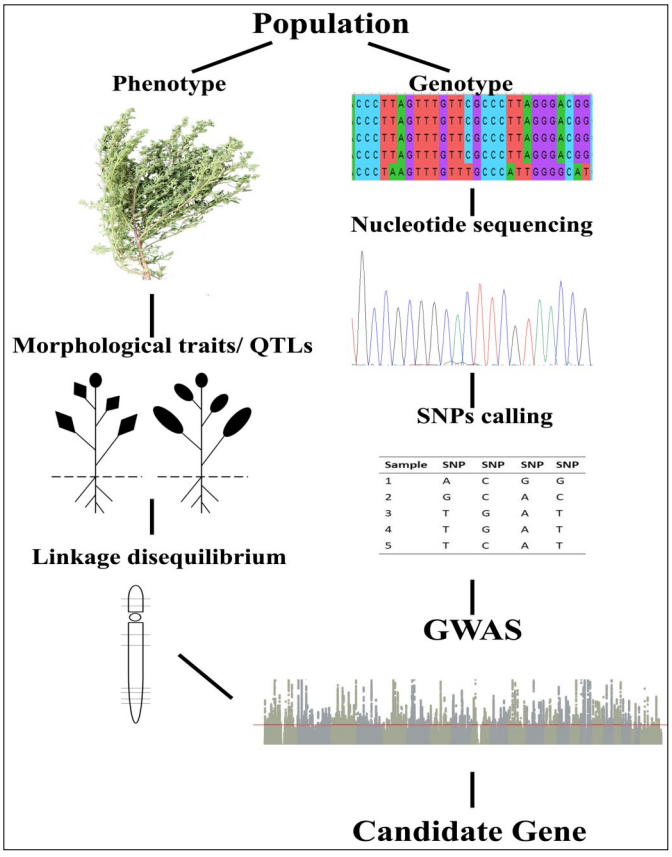

Fig. 1. Representation of general procedure for Genome Wide Associated Studies (GWAS).

analyses on the pre-marker basis are the detection of SNPs which are having a high proportion of missing data, SNPs that have significantly different percentages for missing value in both control and cases, SNPs that depart from Hardy and Weinberg Equilibrium and filtering the SNPs having low minor frequencies (15). Some common statistical methods used for testing the association study at a single locus level are Chi-square test, T-test and ANOVA. ANOVA or t-test can be used when the population has a continuous variable. The odds ratio test is one such test that evaluates the elevated risk of a genotype having an allelic combination of "AA" or "Aa" as compared to a genotype having "aa" genotype by observing the ratio of their odds. Armitage's test can be used for fitting additive genetic models by assuming an ordinal effect (16). Despite efforts to design the study design with proper sample collection, there is always some problem of population stratification at various levels and the effect of population stratification can be detected after post-analysis. One way to visualize such an effect is by Quantile-Quantile (Q-Q) plot where the observed test statistics is being plotted against theoretical distribution value. The presence of possible population stratification is presented by deviation from the diagonal line and its inflation the presence of spurious associations. Simple linear regression will give similar results to t-test and logistic regression with an odd ratio test in a single locus test. Linear mixed model (LMM) is one important and powerful model to control covariation arising from complex correlation structures (17). R packages like GEMMA, EMMAX, or FaST-LMM can be used to analyse LMM or LME2. For binary traits, software packages like GMMAT can be used which are found in the R package which uses a logistic mixed model (18). Family-wise error rate (FWER) or corrected type I error can be used to test the significance level of GWAS to prevent the presence of false positive results and one of the common ways to use Bonferroni correction (19). To visualize and summarize the association signals across the genome, A Manhattan plot is being used. For each tested genetic variant, it plots –log10 (p-value) on y-axis to chromosome position on the x-axis which makes the height inversely proportional to their p-values. Meta-analysis for genome-wide association studies (meta-GWAS) can be used for the identification of functional variants and new gene discovery (20). Some of the open access tools that can be used for conducting GWAS at different stages are – for quality control, packages like PLINK and RICOPILI can be used, for imputation purposes software packages like IMPUTE2, BEAGLE can be used, PLINK2, GEMMA, SNPTEST can be used for association study and software packages like CAVIAR, FINEMAP are used for statistical fine mapping. Sometimes, due to linkage disequilibrium, many non-causal variants are associated with a trait of interest which makes the output of GWAS clustered risk loci (21). One way to validate the result is the use of fine mapping which is in silico process showing potential variants to be causal to target phenotype within the genetic loci based on association statistics and linkage disequilibrium (22, 23). The lead variants with the most significant association are expected to be causal variants, although in some cases it found to be non-causal (24). GWAS can dealing with many characters and different gene-governing traits. Some of the characters may be controlled by more than one gene and some genes might show the presence of epistasis interaction. The presence of exhaustive SNP-SNP interaction followed by multiple hypotheses testing using Bonferroni correction or the Benjamini Hochberg (25) is used. EpiGWAS is one way of detecting the presence of interactions between the target SNP and the remaining genome (26). Despite having great importance, the detection of the presence of epistasis or SNP-SNP interaction in GWAS is challenging. Some of the difficulties it might encounter are- computational burden, the difficulty of replication, the robustness of phenotype, linkage disequilibrium and stringent multiple testing (27, 28). Detecting epistasis can also be done by some statistical methods like LD-based (29), regression-based (30), machine learning approaches (31) and Bayesian approaches (32) and a combination of machine learning and statistical approaches (33). Parametric and non-parametric meta-GWAI strategies could be beneficial to avoid assumptions about genetic effects (34).

Even though the application of GWAS in molecular breeding is quite advanced in many main crops, its application in the Amaranthaceae family is still less. Though recently, some works had already been initiated in the important Amaranthaceae family, still more work needs to be done to dissect and understand all the traits and genes responsible for them for better improvement of the crops under the Amaranthaceae family. So, this review aims to learn the work done so far in different Amaranthaceae families and how we can improve further for a better crop improvement programme.

Comparative analysis of genes identified by GWAS in important Amaranthaceae family

Amaranthaceae family consists of important plants like spinach, *Amaranthus, Chenopodium*, sugar beet and cultivated or wild beet. The plants of the amaranthaceae family are packed with many antioxidants, antimicrobial, antiviral and several medicinal properties for treating different ailments. Also, they are believed to possess resistance to several biotic and abiotic stresses in plants. The most common traits being exploited by GWAS under this family are genes governing stress (both biotic and abiotic stress) and genes for flowering. In the present review, GWAS in some potential crops of Amaranthaceae are discussed and briefly presented in Table 1.

GWAS in Spinach

Spinach is a diploid plant with a chromosome configuration of 2n = 2x = 12 and possesses a medium size genome (35, 36). Enhanced genomic mapping can be used to unlock the understanding of pathogenicity, host resistance for anthracnose and many other non-characterized diseases in spinach. However, limited genomic-disease trait characterization studies have been reported in spinach in the past. Earlier studies on spinach have done for QTL mapping using nine microsatellite markers and 101 AFLP (Amplified Fragment Length Polymorphism) markers for sex linkage study (37). Also, the study was conducted mapping to map the spinach genome related to the disease Stemphylium leaf spot and verticillium wilt using single nucleotide polymorphism (SNP) (38). These SNP-based genomic mapping helped in the identification of association signals of

Table 1. Comparison of genes identified through GWAS in different species of the Amaranthaceae family

Sl.No	Spinach	*Amaranthus*	*Chenopodium*	Sugarbeet
1	Factor SOV6g023690 and SOV4g008150 were reported in regulating flowering time	Candidate genes AH021353-RA, AGL20/ SOC1 and AH021139 were found to promote flowering	CqDODA1 and CqCYP76AD1 involved in Betalaine synthesis pathway	Gene CqCYP76AD1 and CqDODA1 involved in betalain synthesis pathway
2	SpCOL14 and SpFLC related to bolting and flowering	Putative gene AH018224 were associated with early flowering	Candidate genes AUR62011984, AUR62021522 and AUR62016440 function as Sulphate transporter gene	Candidate gene BvSLC35F1-2 and BvACP7 involved in Carbohydrate metabolism
3	NAC domain-containing protein Spo04911 which have its role in regulating plant growth	Candidate genes AH021320, AH001353, AH021553 and AH001354 regulate late flowering	Gene AUR62034957 as putative gene for Salinity tolerance	Candidate gene BvQUA3 (IMABv03g011680) associated with Cell wall biosynthesis
4	Spo04943, a MADS-box factor has function in floral organ development	-	Gene CqGLX2-2 helped in plant growth under abiotic stress condition	Candidate gene BvPGD (IMABv01g018581) and BvTOGT1 (IMABv01g018570), increase sucrose content
5.	-	-	Genes LOC110724999 and LOC110694671 helped plant growth under low phosphorous stress condition	Gene BvLRR (IMABv03g010906), BvDELLA (IMABv09g02 0694) found resistance to Damping off, Root rot and rhizomania

QTL. Recently, physical map LD and haplotype blocking have been used in several studies. SNP tagging to haplotype regions is used for testing the association of qualitative traits with quantitative traits. More LD can be captured between haplotypes and causal variants through haplotype mapping (39, 40). Also, through haplotype blocking and mapping, it can find out epistatic interaction between variants (41, 42). Anthracnose resistance was also studied using GWAS in Spinach by applying pairwise haplotype (htP), single-SNP (sSNP) and multi-marker haplotype (htM) and SNP tagging approaches (43). This study identified a total of 49 significantly associated markers. Of which 13 were identified through the sSNP approach, 24 through the htP approach and 34 through the htM approach. The result indicated the polygenic nature of gene resistance to anthracnose. The genome-wide association of another 20 agronomical traits was studied and identified candidate genes and the associated region with those traits. Homologs of AVP1 and TB1 which regulate plant organ development were reported in spinach. Transgenic lines of different plants show tolerance to several stresses like drought and salinity can survive under low nutrient availability. Some examples are- Transgenic alfalfa (*Medicago sativa*) expressing AVP1 had increased leaf water retention, larger shoot biomass and higher photosynthetic rates than wild type following withheld of watering (44). Tomato plants expressing the AVP1D gene show larger dry weight, improved recovery of shoot growth and higher leaf water potential upon re-watering of the plant (45). Transgenic peanuts and cotton also have greater root biomass with larger shoot systems in dryland fields compared to null segregants (46). Increased shoot growth has been observed in alfalfa, *Arabidopsis*, barley (*Hordeum vulgare*), cotton, rice, creeping bentgrass and peanuts were observed by constitutive expression of AVP1 under saline conditions (47). Also, the same result was found in plants of rice, tomato and Arabidopsis due to overexpression of AVP1 under low P supply soil (48). Other plant characteristics like height, the width of the leaf, petiole, leaf length and leaf width-related genomic components were also identified. An orthologue of the FAR1-related protein of *Arabidopsis* was also found with a β-tubulin gene (SOV6g040410). MADS-box transcription factors SOV6g023690 and SOV4g008150 were also reported in regulating flowering time in spinach (49). Genes SpCOL14 and SpFLC were reported to be related to bolting and flowering. NAC domain-containing protein Spo04911 was reported to have a role in regulating plant growth and stress resistance and the gene Spo04943, a MADS -box factor has a function in floral organ development (50).

GWAS in Amaranthus

The genus *Amaranthus* of family Amaranthaceae has about 70 different species of which 17 are edible. A large collection of *Amaranthus* germplasm was built by the National Botanical Research Institute of India (NBRI) having nearly 400 accessions representing 20 species (51). The crude extract of the plant was reported to be used for the treatment of several ailments due to its nutraceutical properties (52). GWAS analysis of 10 qualitative traits was performed in Amaranth showing the specific association between phenotypes and genetic variants within the genome. The study reported 100 marker-trait associations (MTAs) and 22 MTAs ($P \leq 0.01$, $P \leq 0.001$) on 16 amaranth species and 118 *A. tricolor* datasets respectively on traits like inflorescence colour, branching index and leaf pigmentation ($P \leq 0.01$, $P \leq 0.001$) ($P \leq 0.01$) (53). Gene homolog *AGL20/SOC1* for days to flowering was identified by an interspecies GWAS study (54). In *A. tricolor,* a total of six candidate genes homologous to the flowering time of Arabidopsis *sgs1, bri1*, *fca* and *lba1* were identified through a GWAS study. Candidate gene AH018224 homologous to low-beta-amylase 1(lba1; AT5G47010) of *Arabidopsis* mutant was reported to be related to the function of early flowering (56). Three candidate genes AH021320, AH001353 and AH021553 were also found homologs to an enhancer of FLC, brassino steroid insensitive 1(bri1; AT4G39400) which induces late flowering (55). Another candidate gene AH021139 was reported homologous to sgs1 (AT3G10490) which regulates flowering time through methylation of DNA (56). Also, it was further reported that candidate gene AH001354 is homologous to FLOWERING TIME CONTROL LOCUS A (FCA; AT4G16280) having a function of late flowering in *Arabidopsis* (57).

GWAS in Sugar beet

Sugar beet (*Beta vulgaris* L.) has a chromosome number of 2n =2X= 18 having a genome size of 714 to 758 Mb. The sequence -based genetic study was carried out in sugar beet to find out potential SNP and stress-responsive genes through transcriptome profiling (58-61). Diploid line RefBeet of sugar beet was assembled at chromosome-level assemblies (62).

Candidate genes were homologous to betalain synthesis pathways such as CqCYP76AD1, CqDODA1 and BvDODA1 were reported in sugar beet (63-64). Genome-wide association Studies for genomic and transcriptome analysis of agronomic traits in sugar beet identified 10 candidate genes responsible for disease resistance and five with sugar yield per hectare (65). Candidate genes responsible for pollen fertility were also found through the GWAS study. Putative candidate genes related to sucrose content such as BvMYST1 (IMABv01g023671), BvNR (IMABv01g023663), BvPOD (IMABv01g018569), BvSNAT (IMABv01g018582), BvGN4 (IMABv01g023668), BvPGD (IMABv01g018581), BvTOGT1 (IMABv01g018570), BvCDK12_13 (IMABv01g018584) and BvGBF (IMABv01g018599) were also detected. Genes responsible for carbohydrate metabolism such as the BvSLC35F1-2 gene and gene BvACP7 were also reported. Damping off, Root rot and rhizomania are some important diseases of sugar beet and through GWAS study putative candidate genes related to these diseases are identified. Identified genes are BvCLCN7 (IMABv09g022351), BvEXO1 (IMABv08g027895), BvTSSK6 (IMABv02g031103), BvPRPS (IMABv01g024513), BvLRR (IMABv03g010906), BvDELLA (IMABv09g02 0694), BvFAR1 (IMABv04g006805), BvSERK1 (IMABv09g023194), WRKY1 (IMABv09g020695) and BvPTI1 (IMABv03g010905). Genes related to pollen development were also identified and one important gene BvQUA3 (IMABv03g011680) was found homolog to the homo-galacturonan methyl-transferase gene of Arabidopsis which is involved in the cell-wall biosynthesis (66).

GWAS in Chenopodium

The genus *Chenopodium* of the Amaranthaceae family consists of about 250 different species representing arborescent perennials, colonizing annuals, suffrutescent and herbaceous species (67). Chenopods are known for their rich content of amino acids, proteins, vitamins and minerals (68).An association analysis was performed using 3,156 SNPs in 12 phenotypic traits and found 4 significant MTAs (P< 0.01 for GLM, FDR) (69). Genes controlling saponin content in quinoa were found on chromosome Cq5B. It was harbour between 8.85 Mb to 9.2 Mb of the gene BHLH25 (70).

A study on the copy number variation and single nucleotide polymorphisms in 219 candidate genes in *Chenopodium* accessions having diverse tolerance to salinity identified 15 genes conferring the tolerance of salinity. They found the gene AUR62034957 as closely related to salinity tolerance as it is associated with proline transport. Also, three others quinoa genes AUR62011984, AUR62021522 and AUR62016440 were also found homologs to *Arabidopsis* sulphate transporter gene SULTR1;1, SULTR3;4 and SULTR3;4 (71).

In quinoa seedlings, gene-LOC110724999, LOC110694671 and GDE1 were found to be upregulated in low phosphorus levels and downregulated significantly in exposure to high phosphorus concentrations (72). GWAS study in quinoa reported putative candidate genes for several important characteristics such as Downey mildew tolerance, flower colour, seed saponin content and abiotic stress (73). Two candidates genes CqDODA1 and CqCYP76AD1 were found homologous to genes BvDODA1 (30) and BvCYP76AD1 (29) of the sugar beet which involved in the betalain synthesis pathway. A significant SNP within the gene CqGLX2-2 is found homologous to the *Arabidopsis* gene GLX2-1 encoding glyoxalase enzyme which helps in the growth of plants under abiotic stress conditions (74). A candidate gene for downy mildew resistance was found in region 38.99 - 39.03 Mb of chromosome Cq2A which encodes NBS-LRR (nucleotide-binding site leucine-rich repeat) domain protein having resistance to mildew infection (75).

Conclusion

The Amaranthaceae family is one among the complex family that has many potential species. Plants belonging to this family such as *Amaranthus*, *Chenopodium* and Spinach are used as vegetables/crops, grain, or both. These plants are highly nutritious and at the same time, they are known for their ability against biotic and abiotic stress. GWAS is widely used in deciphering the genetic basis of several complex phenotypes. It served as a foundation experiment to understand the genetic architecture of the trait suggesting potential candidate genes. This method could be used to further explore and identify many other potential genotypic and phenotypic qualities in the family Amaranthaceae for further improvement of the crops. The homologs identified in one species of Amaranthus can be used for other species under the Amaranthaceae family. Through GWAS, SNPs and candidate genes can be identified for several important traits of the plants under this family and they can be used for further research purposes. Many important traits can be targeted in the Amaranthaceae family as they are the source of many nutrients and the fact that they are tolerant to a number of biotic and abiotic factors increases their importance. Understanding and mining genes responsible for the desired characters is important to know the genes or candidate genes that can be identified. The identified candidate genes can further be used for several other crop improvement programs. Understanding the genes present in different species opens up ideas for the introgression of genes from one species to another. The presence of homologs fastened the improvement in different species of crops under this family. As most of the crops under this family come under Neglected and underutilized Species (NUS), exploitation of the crop to understand its molecular basis is still lacking as less research work is being done now, therefore to understand more insightful knowledge on the molecular perspective of the crop, more and more research works needs to be done. Even though research work has started to understand the genes responsible for such important traits, still more and more research work needs to be done for further improvement of the crops under this family.

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Authors' contributions

YL Devi took part in Writing the original draft, conceptualization and Methodology. RJ Devi did project conceptualization, editing, validation, visualization of the final review of the manuscript and funding acquisition. BT takes the role of supervisor of this project.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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