

SSR Fingerprinting of Black Raspberry Cultivars Shows Discrepancies in Identification

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Abstract

Breeding progress in black raspberry (*Rubus occidentalis* L.) has been limited by a lack of genetic diversity in elite germplasm. Black raspberry cultivars are noted for showing very few differences, and seedlings for a lack of segregation for important traits. Genetic fingerprinting using microsatellite, or simple sequence repeat (SSR) markers, can reliably identify unique clones and evaluate diversity in black raspberry cultivars. Twenty-one black raspberry cultivars were sampled from the USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon. Black raspberry clones were also sampled from nurseries, grower's fields, and other black raspberry breeding programs for comparison. These genotypes were compared using 18 polymorphic SSR primer pairs. The black raspberries 'Bristol,' 'Jewel,' and 'Mac Black' had consistent SSR fingerprints between sources. However, plants being sold as 'Black Hawk' and 'Cumberland' had the same fingerprint as 'Jewel'. Plants of 'Bristol,' 'Cumberland,' 'Munger', 'New Logan', 'Plum Farmer' and 'Shuttleworth' in the NCGR collection had identical fingerprints. Eleven unique SSR fingerprints were found among plants being grown or sold as 'Munger', though there was one predominant fingerprint for this cultivar. 'Allen' and 'John Robertson' were each represented by three different fingerprints from three different sources, and 'Earlysweet' and 'Jewel' had SSR alleles at multiple loci that cannot be explained by their reported pedigrees. While overall genetic diversity in black raspberry cultivars is low, discrepancies in the naming of clones appear to be widespread in commercial and research plantings. Future work in this area should focus on sampling additional independent sources of plant material and evaluating clones to determine the extent of performance differences. Further SSR development in black raspberry may be needed to fingerprint some unique clones.

INTRODUCTION

Black raspberry (*Rubus occidentalis* subgenus *Idaeobatus*, $2n=2x=14$) production in the United States is limited by a lack of suitable cultivars. Little progress in breeding new cultivars has been made in the last 40 years because of a lack of phenotypic variation in available germplasm. In recent years, scientists have a renewed interest in black raspberry breeding and gaining a better understanding of genetic diversity in the cultivars. During initial work to document diversity in black raspberry cultivars, Dossett (2011) determined that several cultivars had identical simple sequence repeat (SSR) marker fingerprints in the black raspberry collection at the National Clonal Germplasm Repository (NCGR) in Corvallis, OR. This study follows up on those results by examining the fingerprints of black raspberry cultivars being distributed by nurseries, grown by commercial growers, and in the collections of breeding programs and other researchers.

MATERIALS AND METHODS

DNA was extracted from freshly growing leaf tissue using a modified Puregene kit (Qiagen Inc., Valencia, CA). Leaf tissue was sampled from the cultivars ‘Allen’, ‘Black Hawk’, ‘Bristol’, ‘Cumberland’, ‘Dundee’, ‘Hanover’, ‘Jewel’, ‘John Robertson’, ‘Mac Black’, ‘Munger’, and ‘New Logan’ in the NCGR screenhouse collection as described by Dossett (2011). Black raspberry leaf tissue of one or more of these cultivars was also sampled from five commercial growers in Oregon, three nurseries supplying plants to the industry, the collections of two breeding programs and one other small fruit research program. The purpose of this sampling was to determine the extent of suspected problems and to work with the industry to solve them; participants in this sampling process were therefore promised anonymity in exchange for their cooperation and will not be identified. The 18 SSR primer pairs found to be polymorphic in black raspberry cultivars by Dossett (2011) were amplified in all samples by PCR (conditions described by Dossett, 2011) and separated by capillary electrophoresis using the Beckman CEQ 8000 genetic analyzer (Beckman Coulter Inc., Brea CA). The data were then compiled and analyzed with PowerMarker (Liu and Muse, 2005).

RESULTS AND DISCUSSION

Of the 11 cultivars sampled from multiple sources, plants of ‘New Logan’ and ‘Hanover’ from all sources were ultimately determined to have originated from the NCGR collection. SSR fingerprints of these two cultivars from each source matched those at the NCGR indicating that they were properly labeled. In addition to these, SSR fingerprints of ‘Bristol’, ‘Dundee’, ‘Jewel’, and ‘Mac Black’ were consistent across all sources sampled, including the NCGR collection.

‘Munger’ was sampled from nine different sources and found to have 11 different SSR fingerprints. This included multiple SSR fingerprints from two separate nurseries, and six different SSR fingerprints from six plants sampled from one grower. One of these clones matched ‘Jewel’, another matched one of the clones being distributed by a nursery, and the others were unique. While other growers had multiple SSR fingerprints for ‘Munger’ in their fields, most of the ‘Munger’ sampled from nurseries and black raspberry growers ($n=30$) had a single SSR fingerprint that also matched that of ‘Munger’ in the NCGR collection. This fingerprint also matched ‘Bristol’ from every source, as well as ‘Cumberland’, ‘New Logan’, ‘Plum Farmer’ and ‘Shuttleworth’ at the NCGR. Three of the unique ‘Munger’ clones sampled each differed from this fingerprint at single loci (Rubus 270a, Rub1C6, and RhMe007aB01, respectively, see Table 1), supporting the possibility that these could be somatic variants that have been propagated over the years.

‘Cumberland’ and ‘Black Hawk’, distributed by one of the nurseries sampled and then subsequently grown and evaluated in breeding plots, were found to match the SSR fingerprint of ‘Jewel’, indicating that other sources for these cultivars should be sought and tested for comparison. ‘John Robertson’ and ‘Allen’ were also sampled from three different sources and each was found to have three unique fingerprints. Relationships of the different black raspberry clones identified in this project are illustrated in Figure 1.

SSR fingerprinting identified discrepancies in names as well as discrepancies in reported pedigrees. For example, there was broad consensus between sources on the identities of ‘Bristol’, ‘Dundee’, and ‘Jewel’. Despite this, the SSR fingerprint for ‘Jewel’ does not match its reported pedigree ($[(\text{‘Bristol’} \times \text{‘Dundee’}) \times \text{‘Dundee’}]$). At three SSR loci (ssrRhCBA23, Rubus 110a, and Rubus 275a), ‘Jewel’ had alleles that cannot be explained by its parents (data not shown). Similarly, none of the three ‘Allen’ clones identified had alleles that matched the reported pedigree of this cultivar ($\text{‘Bristol’} \times \text{‘Cumberland’}$). These differences, and others, are highlighted by Dossett (2011). Unfortunately, limited availability of some of the cultivars and a lack of consensus in the identities of others, make it difficult to speculate whether an incorrect identity of the parents, progeny, or both, are the reason for mismatched pedigrees.

Dossett (2011) found black raspberry cultivars to be very closely related to each other and found little genetic diversity between them in comparison to wild accessions.

Despite the differences found between black raspberry cultivars, the same alleles are present at the loci sampled, and all of the identified clones clustered with other black raspberry cultivars in the grouping identified by Dossett (2011). From this perspective, new genetic diversity should be broadened to insure sustained breeding progress; however, the discrepancies noted are still important to the research community. Because nurseries distribute multiple clones of the same name, researchers cannot reliably obtain uniform plants or have true comparisons for cultivar or breeding trials even when using only a single supplier. This issue will need to be resolved before researchers can reliably compare data on black raspberry performance and pedigrees on new selections in breeding programs.

CONCLUSIONS

Mislabeled black raspberry cultivars appears to be a widespread problem affecting nurseries, growers, and researchers. While there appears to be consensus on the identity of some important cultivars (e.g. 'Bristol', 'Jewel' and 'Mac Black'), the identity of other cultivars (e.g. 'Allen', 'John Robertson' and 'Munger') is less than clear. Further sampling from additional sources for these cultivars may help to identify which clones are predominant. However several of these cultivars (e.g. 'New Logan', 'Plum Farmer', and 'Shuttleworth') are no longer widely available and may have been lost. Phenotypic evaluation of different clones in a common environment is needed to identify the best performing clones of the same name, as well as to determine if there are differences between clones with identical fingerprints and different names. The level of polymorphism among black raspberry cultivars in existing SSR markers is very low and additional markers may be needed in the future to reliably differentiate between different clones that currently have the same SSR fingerprint. Ideally a foundation nursery that contains true-to-type genotypes, which could provide nurseries with nuclear stock, will be established to help reduce the problem of cultivar misidentification in the black raspberry industry.

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Literature Cited

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Tables

Table 1. Differences in SSR fingerprints of 11 different clones of ‘Munger’. For simplicity, samples with matching fingerprints have been removed so that only a single representative of each fingerprint is listed. Loci for which no variation was observed between different ‘Munger’ clones are not shown. The first fingerprint listed represents the clone at the National Clonal Germplasm Repository in Corvallis, OR as well as the majority of industry plants sampled. Differences from this fingerprint are shown in bold.

Genotype	Rubus26a	Rubus126b	Rubus275a	Rubus270a	ssrRhCBA23	Rubus110a	RubIC6	RhMe013cE02	RhMe007aB01
Munger NCGR	139/141	154/168	116/144	163/165	124/126	183/185	258/268	320/322	147/151
Munger_N1_E	139/141	154/168	116/144	163/165	124/126	183/185	258/268	320/322	147/147
Munger_G5_D	139/141	154/168	116/144	163/163	124/126	183/185	258/268	320/322	147/151
Munger_G5_F	139/141	154/168	116/144	163/165	124/126	183/185	258/258	320/322	147/151
Munger_B1_R47	139/141	154/154	116/144	163/167	124/126	171/183	237/268	322/322	147/151
Munger_B2	139/139	154/168	116/144	163/165	124/126	183/183	268/268	322/322	147/151
Munger_G4_A	139/139	154/168	116/144	163/163	124/126	183/183	258/268	320/322	147/147
Munger_G5_A	139/143	158/168	144/144	163/163	112/124	169/185	258/268	320/322	147/151
Munger_G5_C	139/141	154/168	144/144	163/163	124/124	183/183	258/268	320/322	147/147
Munger_G5_E	139/139	154/168	144/144	163/165	124/124	183/183	258/268	320/322	147/147
Munger_N1_D	139/139	154/168	144/144	163/163	124/124	183/183	258/268	320/322	147/147

Figures

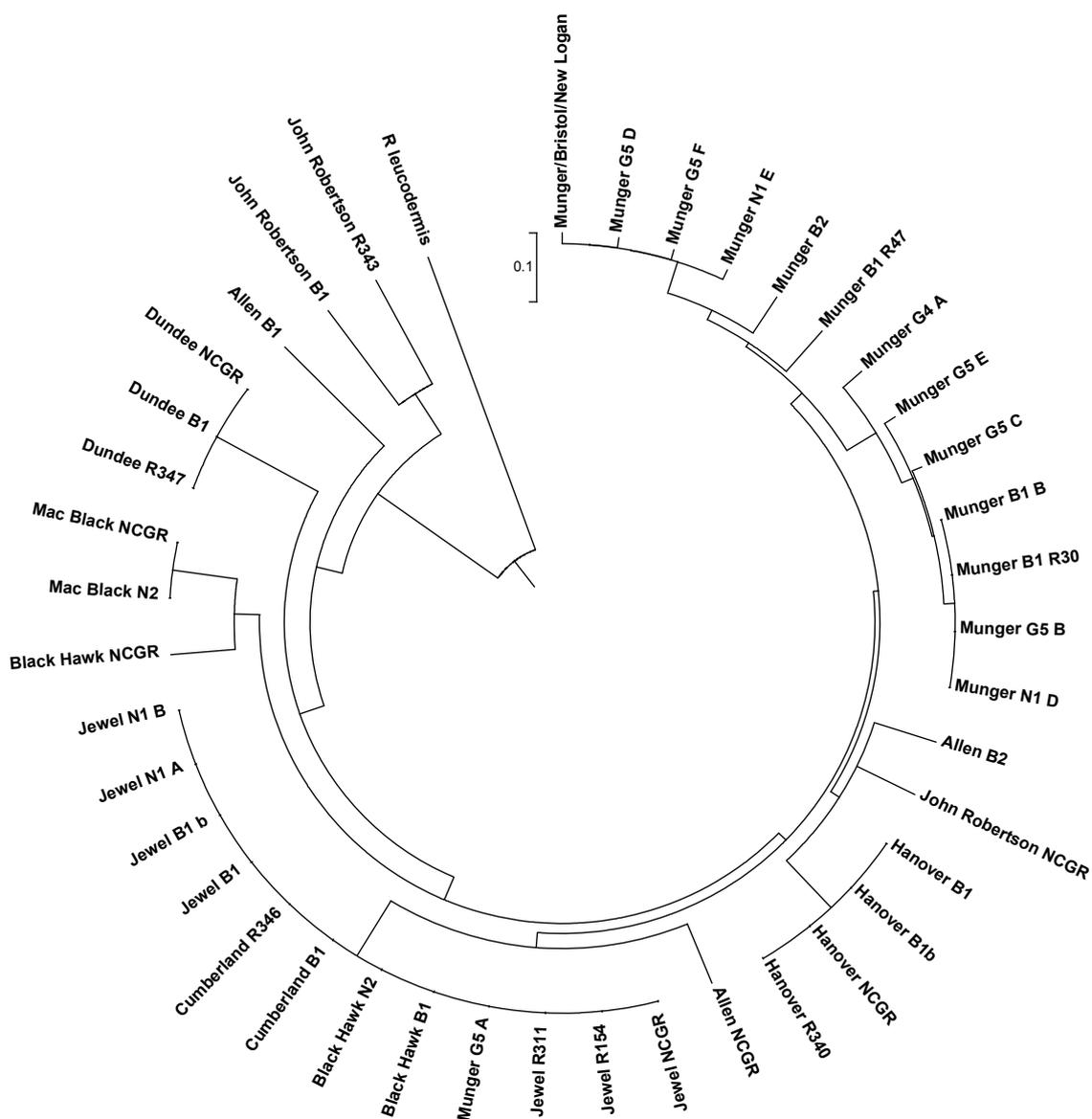


Fig. 1. UPGMA dendrogram depicting relationships of black raspberry accessions sampled. Samples from the National Clonal Germplasm Repository in Corvallis, OR, USA, have “NCGR” following the cultivar name; other accessions have been coded to maintain anonymity of sources. Collapsed branch on dendrogram labeled “Munger/Bristol/New Logan” contains accessions of ‘Munger’ ($n=30$), ‘Cumberland’ from the NCGR, and all accessions of ‘Bristol’ ($n=7$), and ‘New Logan’ ($n=4$). A single accession of *R. leucodermis* Gray ex Torr. has been included to root the dendrogram.

