A Cost–Benefit Analysis of DNA Informed Apple Breeding

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Abstract. **Incorporating DNA-informed breeding techniques can improve selection efficiency for desired traits as compared with conventional breeding methods that do not use DNA-informed techniques. Incorporation of DNA technologies requires additional costs associated with reagents, equipment, and labor. To elucidate the cost-effectiveness of DNA-informed breeding in perennial crops with multiple years per generation, we conducted a cost–benefit analysis examining incorporation of marker-assisted selection (MAS), a type of DNA-informed breeding, applied to an apple breeding program. Annual operational costs for a midwest apple breeding program were used to develop a simulation with inputs including itemized costs and per unit costs for procedures at each breeding program stage. Simulations compared costs of MAS breeding techniques to conventional breeding methods to identify the break-even point (BEP) where cost-savings associated with MAS equals the accrued additional costs. Additional sensitivity analyses were conducted to examine changes in laboratory costs, seedling maintenance costs, and seedling evaluation costs. We found the BEP for this program occurs when MAS results in a removal rate of 13.18%, and changes to other costs (i.e., maintenance costs) result in a smaller percent decrease to the overall program budget. Our findings are useful to perennial crop breeding programs in which managers are considering incorporating DNA-informed breeding techniques.**

The Rosaceae family composes nearly 43% of the United States per capita noncitrus fruit consumption by weight. This amount translates to a value of over 7 billion dollars for fresh and processed rosaceous products annually (Iezzoni et al., 2010). The per capita consumption of these fruit crops has slowed in recent years and in some instances has even decreased (Gallardo et al., 2015). To counter this stagnation, breeders seek to develop breakthrough cultivars that exhibit a suite of improved traits to capture the interest of both producers and consumers (Yue et al., 2013). Ideal fruit characteristics for producers and consumers often differ, making the task of breeding for both targeted audiences challenging (Gallardo et al., 2015; Yue et al., 2012.

Advances in DNA technologies for crop improvement have increased breeders' ability to adapt their selection processes to meet dynamic market demands. One such DNA technology is marker-assisted selection (MAS), which allows breeders to predict the presence or absence of target traits based on association with known DNA segments (Frey et al., 2004; Johnson, 2003). MAS techniques allow for selection of quantitative traits controlled by many genes in a relatively shorter time than conventional breeding methods (Knapp, 1998; Moreau et al., 1998; Ru et al., 2016). Previous research conducted for various crops has shown the superiority of MAS techniques for breeding. Such crops include almond (Sorkheh et al., 2017), apple (Edge-Garza and Peace, 2009; Kenis et al.,

2008; Kumar et al., 2012; Tartarini et al., 1999; Urbanietz et al.,1996), cassava (Rudi et al., 2010), common bean (Schneider et al., 1997), maize (Lande and Thompson, 1990; Morris et al., 2003; Stromberg et al.,1994), pome fruit (Tartarini and Sansavini, 2002), rice (Suh et al., 2013), stone fruits (Edge-Garza et al., 2009; Testolin, 2002), and wheat (Kuchel et al., 2005). Marker tests developed for apple include tests for scab (Tartarini et al., 1999), tree habit (Kim et al., 2003; Morimoto and Banno, 2015), skin overcolor (Zhang et al., 2014), acidity (Xu et al., 2012), and firmness (Baumgartner et al., 2015) among others (Evans and Peace, 2017). DNA marker tests have been used in the University of Minnesota program to screen for acidity, cell wall proteins, and disease resistance.

Other studies examine the economic impact of MAS adoption by fruit breeders beyond academia, which has been limited due to technical and economic barriers (Evans et al., 2012; Peace, 2017; Ru et al., 2015). Primary barriers include developing accurate DNA tests for high value traits and creating access to testing facilities for breeders. Secondary barriers are determined by breeder resources and whether capital and labor can be allocated to new procedures. To circumvent these barriers, breeders need to be able to implement a DNA testing plan with the available monetary resources, and to continue operating each year (Ru et al., 2015). Without knowledge of cost-efficient implementation schemes, breeders risk misallocating resources without achieving their breeding goals.

A study of potato conducted by Slater et al. (2013) concludes that an optimal relationship between the effectiveness of using markers and selection intensities in MAS can be identified. They found application of markers in the second clonal generation achieves maximum cost-efficiency in potato due to the high cost of phenotyping in the subsequent stages. Further studies in rice show cost-effective MAS is achievable while developing salt tolerant rice varieties despite the additional costs incurred (Alpuerto et al., 2009). MAS cost-effectiveness results from reducing the amount of time to cultivar development and the increase in potential revenue due to improved varieties better suited to variable growing conditions.

To our knowledge, only two studies have examined the economic impacts of MAS in horticultural breeding programs such as apple, grape, and strawberry. One study identified components of breeding programs that enable the incorporations of cost-efficient DNA testing (Luby and Shaw, 2001). This study indicated that inheritance of the trait, timing of trait expression, application timing of DNA testing in a program, and testing costs play important roles in determining cost-efficient MAS. A second study by Edge-Garza et al. (2015) developed a calculator tool examining one type of MAS called marker-assisted seedling selection (MASS). MASS requires tissue samples be taken at immature growth stages in a plant's juvenile

phase and that these samples be evaluated for traits through marker testing, which allows rapid screening (Afanador et al., 1993; Collard and Mackill, 2008). Early detection using DNA marker tests improves selection by removing offspring with or without the DNA marker before the plant physically displays these characteristics (Liebhard et al., 2003; Urbanietz et al., 1996). Early detection thus reduces time to selection by detecting the alleles with the targeted trait early in the plant's life. Early screening is especially valuable in perennial fruit crops where conventional breeding relies on observation of fruit qualities requiring multiple years of growth before selection occurs (Hancock, 2008; Janick and Moore, 1996). Examining removal rates, Edge-Garza et al. (2015) determined that application of MASS as early as possible was not necessary as long as MASS occurred before additional labor costs are incurred from seedling handling (i.e., planting in orchards and maintenance).

The decision support tool developed by Edge-Garza et al. (2015) examines the MASS application at the early stages of the breeding program. However, efficient DNA testing can occur at multiple stages in the breeding program (Xie and Shizhong, 1998). The dynamic nature and long breeding cycle of apple distributes activity costs over years, but the exact number of years varies among programs and hence challenges development of cost-savings calculators for each unique case. This study develops and applies a dynamic cost estimation tool to examine impacts from technology changes, such as MAS application, in the University of Minnesota (UMN) apple breeding program. We examined costs during a single year of the program for which MAS is applied and constructed a decision support tool with a dynamic view of the entire breeding program over multiple years and stages for MAS usage. The breeding program is examined from the crossing stage to the cultivar release stage.

Materials and Methods

A simulation model having the University of Minnesota apple breeding program as the basis was developed to examine the costs associated with different stages in the breeding program over ≈30 years. The model used itemized costs incurred in 2015 by the apple breeding program in reference, breeding records from the previous 20 years, breeder interviews, and a spreadsheet-based questionnaire. These resources were used to develop a detailed timeline of cultivar development from parental selection to cultivar release in an Excel spreadsheet model.

Costs were collected for the main breeding stages: crossing, greenhouse, seedling test orchards, clonal test orchards, and pre-commercialization orchards (Fig. 1). The costs for each stage were further broken down as labor costs and consumable costs for various processes. The crossing stage processes include germplasm maintenance, parental cross planning, and seed extraction.
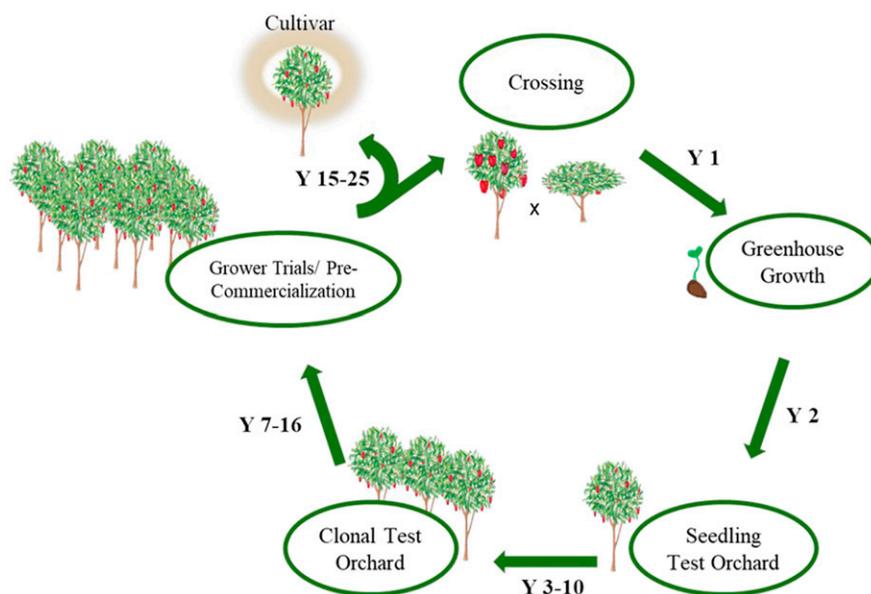

Fig. 1. Apple breeding program diagram following a single cohort of crosses through to selection release as a cultivar between years (Y) 15 and 25 and reentry into the program as a parent. Crosses are made in spring of the first season with seed collection at the end of the same season. Seeds are germinated in the greenhouse and then planted at the end of the second season. Seedlings remain in seedling test orchards for years 3 to 10. During this time selections are made as to which advance to clonal test orchards. Selections remain there for years 7 to 16, during which time advanced selection occurs. Advanced selections then are moved to grower trials and pre-commercialization, where they remain for years 15 to 25 in the program; after which, the cultivar is released and used as parental material for future apple breeding.

The greenhouse stage consists mainly of seed germination, container production of seedlings from the crosses, and removal of weak individuals. Seedling test orchards processes consist of the asexual propagation of each seedling by bud grafting to a single dwarfing rootstock. Evaluation of individuals for fruit quality typically begins in the fourth to fifth year after budding when individuals begin bearing fruit within a planting. Due to the prolonged juvenile phase in fruit tree crops, a distribution of fruit-bearing individuals occurs over the next 5 to 6 years as increasing numbers of individuals reach maturity. Undesirable individuals are removed from seedling test orchards as they are evaluated. Desirable individuals in seedling test orchards then are moved to clonal test orchards, which consist of multiple clones grafted onto dwarfing rootstocks at a second location from the seedling test orchard. Another round of selection and removal occurs when some clonal test orchard selections are chosen for potential cultivar release and enter pre-commercialization orchards, which are comprised of multiple asexual propagations in multiple test orchards around the state or country. For each stage of test orchards, itemized costs were estimated for establishment, maintenance, and fruit evaluations.

Costs collected for MAS include tissue collection, DNA extraction, application of markers, interpretation of DNA test results, and implementation of removal based on test results. After obtaining MAS cost data, the following three assumptions were made to develop models for MAS for this program. 1) Markers with close linkage to a trait of interest and low failure rate due to technical issues exist for the purpose of selecting individuals in a breeding program. 2) The cost of marker development, while not included in this model, is recognized as a substantial cost that may be offset by inputs from the public sector such as RosBREED, which brought together collaboration aimed at improving DNA-informed breeding in rosaceous crops (Dreher et al., 2003; Edge-Garza et al., 2015). 3) Markers do not reduce the number of selections made in a program.

In an average year, land and labor resources in the breeding program at the seedling test orchard can support the production of about 5000 to 6000 seedlings. Achieving the goal of 30,000 seedlings in seedlings test orchards requires about 5 to 6 years, with new crosses each year within the program (Kellerhals et al., 2007). Repeated crossing increases the time demand for seedling production, which means that producing a cultivar requires repetition of yearly itemized costs. As time progresses, the repetition of crosses and breeding program procedures begin to overlap (i.e., seedlings from previous years are still growing as new seedlings are planted). This overlap creates a combination of costs for the program as various procedures are conducted simultaneously in a growing season.

Removal rates and selection rates of seedlings in the seedling test orchard were incorporated into the model to simulate real world situations. Records of removal rates and descriptors provided an established history and descriptions of how quickly seedlings are removed following evaluations. Based on

removal records, it is assumed that removal rate accounts for ≈90% of the total seedlings under evaluation in a year. This assumption allows for the modeling of the distribution of trees bearing fruit over time, and modeling of the timing when trees are evaluated and removed. Evaluations continue for the remaining 10% of the seedling population until they are discarded or selected. The seedling test orchards removal rates were the average rates over the previous 6 years for the apple program, where the removal rates were recorded for individual tree blocks planted at about the same time. These removal records indicate an estimated 0.5% of stage 1 seedling cohorts continue to the clonal test orchards.

After individuals enter clonal test orchards, the number of years before they are selected to enter pre-commercialization ranges randomly from years 9 to 16 in the breeding program, based on the program records. Therefore, in the model, the year of selection for the cultivar release is distributed over this time based on the number of individuals entering clonal test orchards, and the total number of individuals within a year of clonal test orchard evaluation. As the number of individuals increases the size of the selection pool, the likelihood of selection increases until the maximum number of individuals a program can maintain per year is achieved.

After the steps just discussed were taken to develop a conventional model, a model was developed to simulate the implementation of MAS at the greenhouse stage. The number of seedlings produced each year remained constant, with varying rates of individuals being removed. The rates of removal were estimated at high (50%), medium (25%), and low (10%) to explore how various removal rates affect the costs. These percentages reflect the percentage of tested individuals that are removed from the program, and not the percentage of total initial seedlings removed. Removal rates were chosen based on breeder feedback and review of achieved levels of MAS in the current program for tested seedlings. Though 10% does not follow Mendelian trait inheritance, the need to identify the BEP required more stringent removal because the BEP was still surpassed at 12.5% removal.

Because the program needs to produce 30,000 seedlings to identify a marketable cultivar, and because the breeding programs capacity is 6000 seedlings per year, the model simulates 5 continuous years of seedling production in the greenhouse stage (that is, the model simulates the time and costs it takes the program to generate enough seedlings to release a cultivar), with DNA tests run on 3,000 of the individuals. Extended and overlapping juvenile phases of these individuals results in clonal test orchard activity costs over 18 years from years 7 to 25. For example, if an individual is selected at year 9 in seedling test orchards, it would enter clonal test orchards in year 11 and remain there for 8 to 10 years of additional evaluation. That process indicates the individual would be removed in year 20 or 21. The simulation was repeated 50 times, and then

the simulated clonal test orchard costs results were averaged.

Sensitivity analyses. The costs of labor and MAS technology constantly change and may vary across different breeding programs. To test how sensitive our model results are to changes in costs for labor and consumables, we conducted sensitivity analyses. Crossing and greenhouse stage costs were held constant, as they are upstream of MAS application. Costs for MAS technology and labor for the seedling test orchards were varied to examine their impact on the efficiency of MAS in the breeding program. Clonal test orchard parameters were held constant under the assumption that MAS would not change the likelihood of producing a cultivar. The effects of MAS were observed in the seedling test orchard by varying parameters such as laboratory costs of MAS, labor cost for seedling maintenance, and labor cost of seedling phenotyping. When MAS is applied for the sensitivity analyses, the removal rate is held constant at 50% to create more realistic scenarios based on breeding program records. The lower removal rate MAS scenarios have greater potential for cost savings through reduction of seedling maintenance

and evaluation costs as they have higher numbers of seedlings. Observed trends at the 50% removal rate are therefore assumed representative for lower removal rate scenarios.

The first sensitivity analysis examined the effects of decreased laboratory costs for MAS (i.e., lower reagent costs, more automation, or more efficient workers, etc.), ranging from 10%, 25%, and 50% cost reduction based on breeder inputs The second sensitivity analysis explored the impacts of reduced labor inputs for maintaining seedling test orchard seedlings due to increased efficiency of routine maintenance practices. Maintenance efficiency was estimated at 2%, 5%, and 10% reduction of labor inputs for both the conventional breeding model and MAS model based on perceived feasible changes by breeders. The third sensitivity analysis examined the effects from reduced labor costs for seedling test orchard yearly evaluation due to theoretical increased efficiency from new phenotyping technology. The evaluation efficiency estimation considered reduction of 10%, 15%, and 20% for labor inputs in both the conventional breeding and the MAS models based on theoretical technological
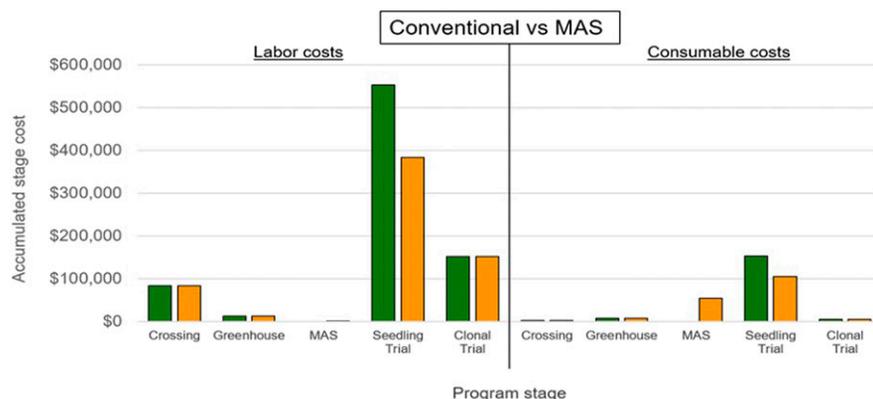

Fig. 2. Conventional (green bars) vs. marker-assisted selection (MAS; orange bars) costs broken down into labor and consumables (i.e., fertilizer, pesticides, etc.) for an apple breeding program. Seedling trials comprise ≈73% of total program costs over a period of ≈30 years.
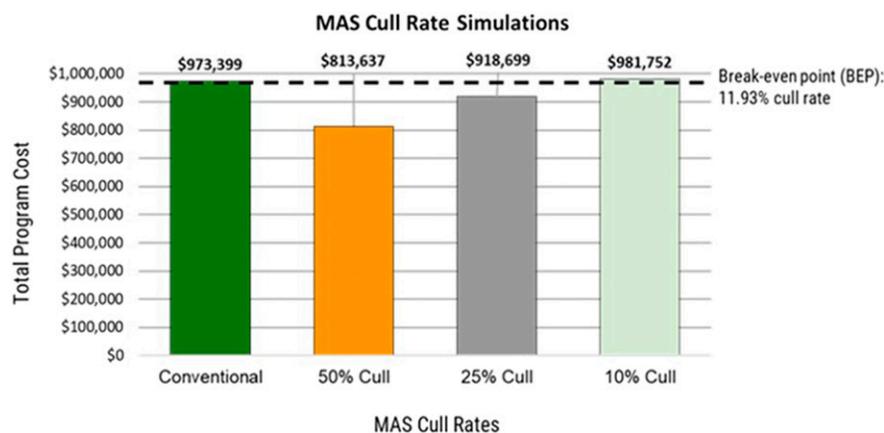

Fig. 3. Three removal rates were modeled to explore marker-assisted selection (MAS) impacts on total apple program costs and to determine break-even point (BEP) at which the additional cost of MAS equals the cost-savings generated. The BEP was achieved with an 11.93% removal rate.

improvements for improving evaluation performance.

Results

Conventional breeding. The conventional breeding model describes the costs of planting and examining seedlings for the duration of the entire program without any MAS at the greenhouse stage. Most costs are associated with labor and occurred during years in seedling test orchards (Fig. 2). Per unit seedling costs varied greatly across stages. Seedlings up to the greenhouse stage cost about $1.18 per seedling. Maintenance and establishment for both seedling test orchards and clonal test orchards result in a significant increase in the cost per seedling at each stage. Seedling test orchard establishment (i.e., grafting onto rootstock planted in the orchard) cost is about $7.00 per seedling, with maintenance at $4.00 per seedling for each additional year. Clonal test orchard establishment cost is about $25.22 per tree for nursery costs, with each additional year costing about $63.25 for maintenance and evaluation. The total cost of breeding and selecting a single cultivar for pre-commercialization using conventional breeding is about $973,399 (Fig. 2). Of this dollar amount, labor costs account for 82.6% ($803,924), and consumable costs account for the remaining 17.4% ($169,474) (Fig. 2). Seedling test orchards alone account for 72.7% of the total costs of conventional breeding, contributing 68.9% ($554,121) of total labor costs and 90.4% ($153,198) of total consumable costs.

Downstream MAS. Initial seed production and germination are held constant to examine downstream results of increased removal at the greenhouse stage due to the use of MAS. The MAS models examine the reduced number of individuals entering seedling test orchards based on marker information with three removal rates during greenhouse growth: low (10%), medium (25%), and high (50%). The costs for tissue collection, DNA extraction, and using markers are included in the three MAS models in addition to the costs incurred by the conventional breeding practices. The cost per individual for greenhouse, seedling test orchard establishment and maintenance, and clonal test orchard establishment and maintenance remain the same as in conventional breeding. The number of individuals entering clonal test orchards were proportionate to the number of individuals in seedling test orchards in Fig. 3. The simulation results show the cost of implementing MAS at the low removal rate incurs greater costs than conventional breeding methods. MAS became more cost-efficient at both the medium and high removal rates. The model was run holding the number of individual plants in the crossing stage, the greenhouse stage, and advancements from seedling test orchards to clonal test orchards constant (Fig. 3). The simulation results indicate that the medium and high removal rates were cost efficient, while the low removal rate was not. This information indicates that the break-

even removal rate (the removal rate where the cost savings equals the additional cost incurred from using MAS) for the application of MAS at the greenhouse stage of production lies between 10% and 25%. For the high MAS (50%) removal rate, labor costs account for 74.9% ($645,834) of the total costs, and consumable costs account for the remaining 25.1% ($216,628) of total costs (Fig. 3). Seedling test orchards represent 58.6% ($505,893) of total costs, with 61.4% ($396,239) due to labor and 50.6% ($109,654) due to consumables.

Break-even point. A break-even point informs breeders at what cost or removal rate MAS becomes cost-efficient and when
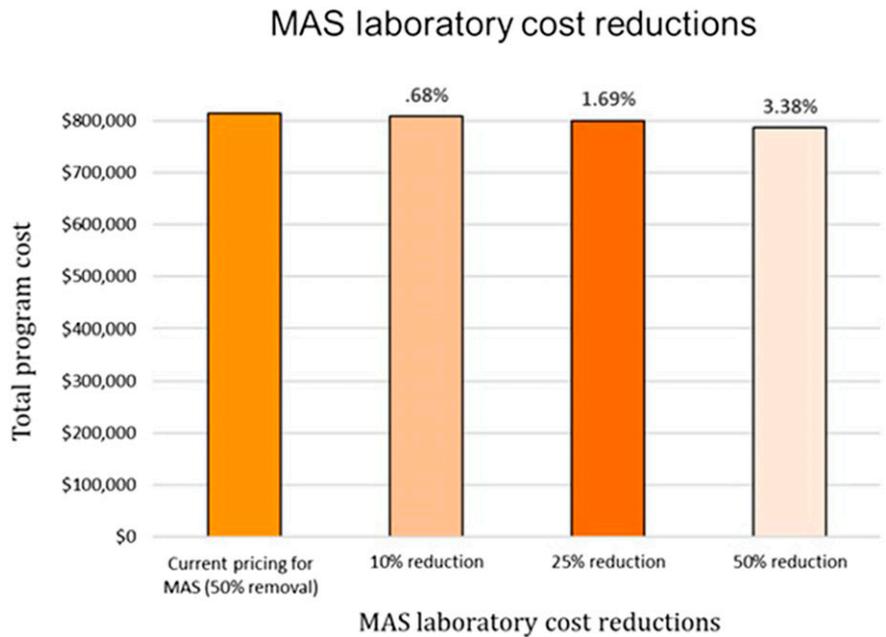
Fig. 4. This chart compares current pricing for marker-assisted selection (MAS; far left) to three levels of cost reduction for MAS laboratory costs over a period of ≈30 years in an apple breeding program. Each simulation presented assumed 50% of individuals undergoing MAS were removed.
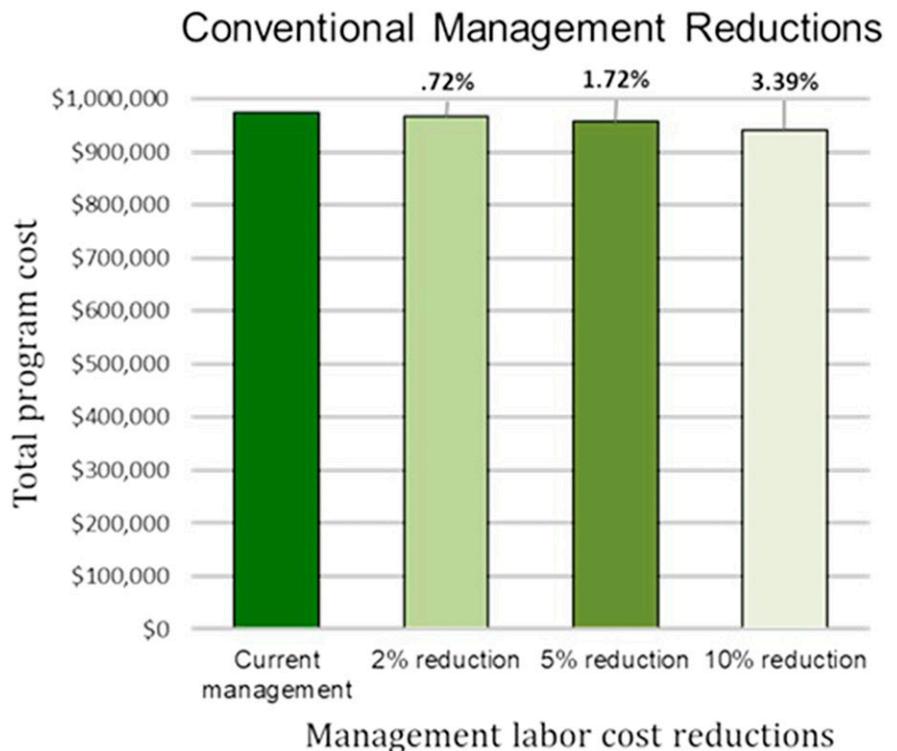
Fig. 5. Sensitivity analysis (2) examining reduction of stage 1 seedling management labor costs in conventional apple breeding. Cost reduction levels are 2%, 5%, and 10%, with costs calculated over 5 years of crossing. MAS costs are excluded from conventional breeding simulations.

practices are no longer cost efficient. The break-even point was obtained by holding all stages constant except seedling test orchards. The break-even removal rate for MAS implementation is ≈11.93%, with a total cost of $973,398.31 (Fig. 3). A lower removal rate using MAS will result in higher costs to the breeder compared with conventional breeding methods.

Sensitivity Analysis 1. The sensitivity analysis compared scenarios where DNA testing costs for MAS decreased by 10%, 25%, and 50%, resulting in a removal rate of 50% (Fig. 4). A 50% reduction in DNA testing costs results in the largest cost savings over 25 years ($38,080) when compared with the 2015 costs of DNA testing. Additionally, the results indicate that MAS is cost effective at both 10% and 25% MAS laboratory cost reductions, with a cost savings of $7616 and $19,040 respectively. Compared with the conventional breeding model, a 50% MAS laboratory cost reduction results in an estimated cost savings of $300,974; a 25% reduction results in a cost savings of $281,934; and a 10% cost reduction means a cost savings of $270,510 (Fig. 4).

Sensitivity Analysis 2. Decreasing the cost of seedling management by 10% resulted in a 3.4% ($32,968) reduction in total costs for conventional breeding methods (Fig. 5). The 10% seedling management cost reduction resulted in a total cost of $940,431. This remains a greater cost than MAS, with 50% removal rates achieved in the breeding program. Decreasing seedling management costs by 10% in the MAS model resulted in only a 2.3% decrease ($16,485) in total costs for MAS breeding methods. A 2% and 5% reduction in seedling management costs for the MAS model resulted in 0.5% ($3486) and 1.2% ($8361) cost reductions, respectively (Fig. 6).

Sensitivity Analysis 3. Seedling evaluations require high labor inputs in the breeding program. Decreasing the cost of labor for evaluations by 20% results in a 3.3% ($32,375) reduction in the total cost (Fig. 7). Evaluation labor cost reduction by 10% and 15% resulted in 1.6% ($15,833) and 2.5% ($24,104) cost decreases, respectively, for the conventional model. A 20% decrease in evaluation labor cost in the MAS model resulted in a 2.4% ($16,699) reduction in total cost. Reducing evaluation labor in MAS by 10% and 15% results in 1.2% ($8428) and 1.8% ($12,563) cost reductions, respectively (Fig. 8).

Discussion

MAS has the potential to reduce costs incurred by the breeding program or to improve the program's economic efficiency. One barrier to the implementation of a new technology, such as MAS, is an absence of decision support tools that inform breeders about internal costs and benefits of using the new technology (Dreher et al., 2003; Ru et al., 2015). Internal cost savings could result from greater removal of inferior seedlings,

reducing labor costs, or reducing the time to release by early identification of superior individuals. We developed a tool, customizable for use by specific programs, that estimates the financial costs to a program of implementing new technologies, such as MAS. Detailed information about potential costs increases the likelihood of programs
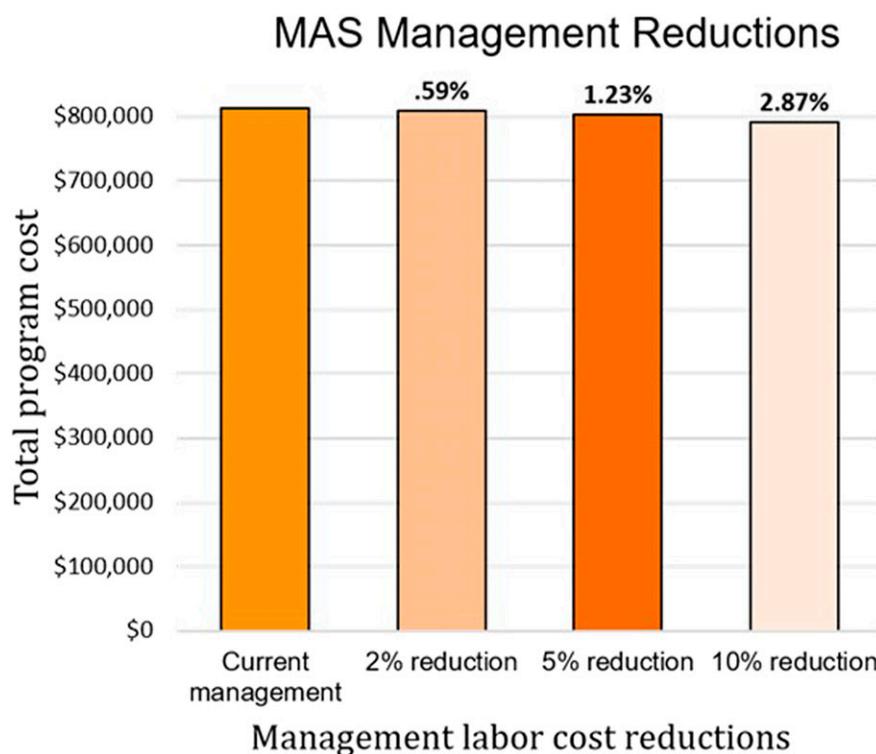

Fig. 6. Sensitivity analysis (2) examining reduction of stage 1 seedling management labor costs in marker-assisted selection (MAS) for an apple breeding program. Cost reduction levels are 2%, 5%, and 10%, with costs calculated over 5 years of crossing.
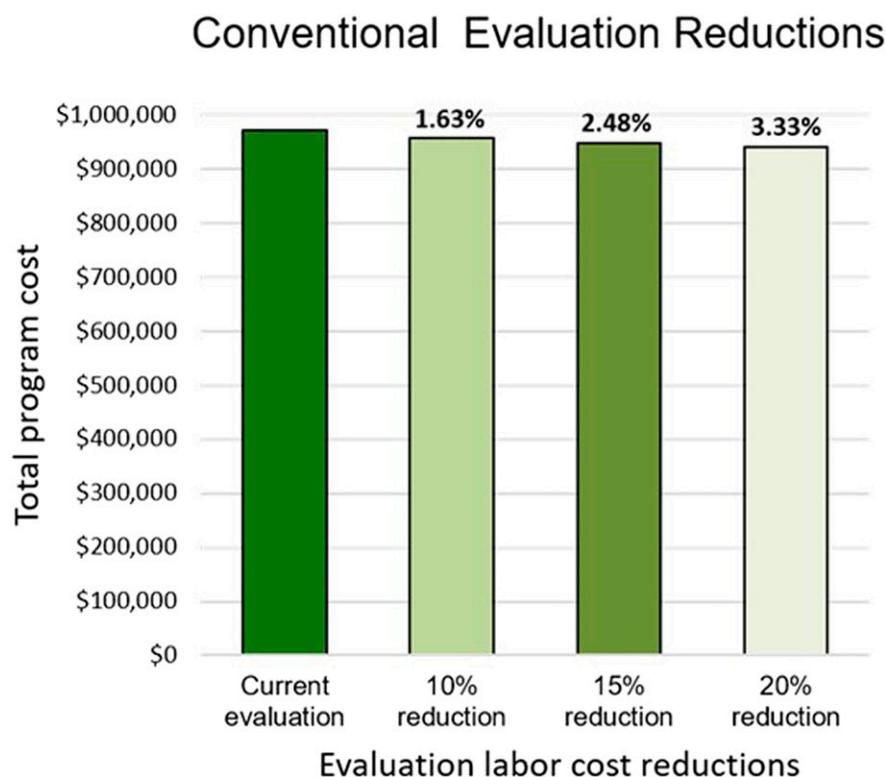

Fig. 7. Sensitivity analysis (3) examining the reduction of stage 1 seedling evaluation costs in conventional apple breeding. Evaluation cost reductions are 10%, 15%, and 20%, with costs calculated over 5 years of crossing. MAS costs are excluded from conventional breeding simulations.
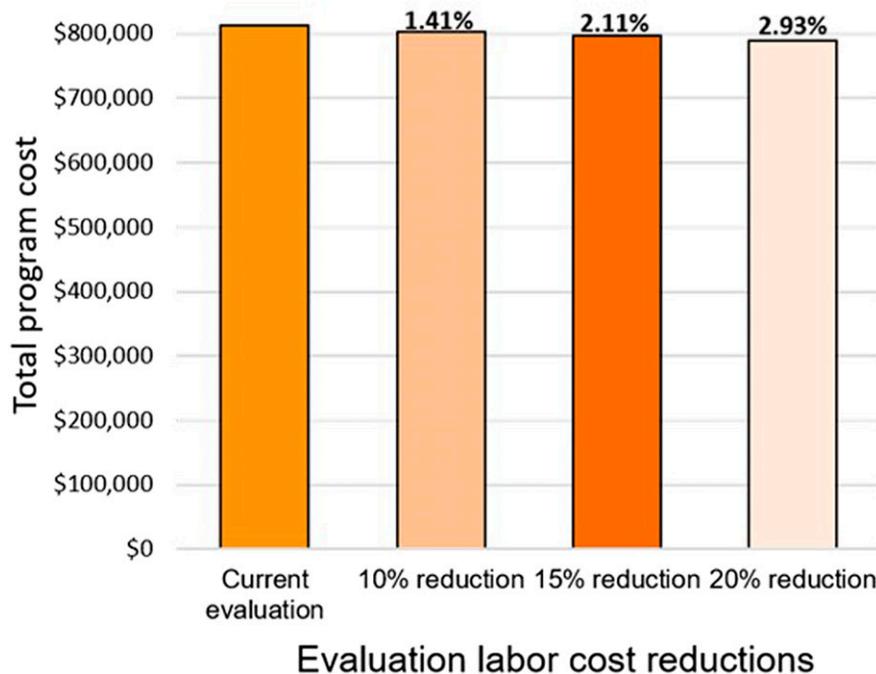
MAS Evaluation Reductions

Fig. 8. Sensitivity analysis (3) examining the reduction of stage 1 seedling evaluation costs for marker-assisted selection (MAS) in an apple breeding program. Evaluation cost reductions are 10%, 15%, and 20%, with costs calculated over 5 years of crossing. MAS costs are excluded from conventional breeding simulations.

incorporating the technology. Even with factors such as trends of decreased costs for DNA technology, increased high throughput phenotyping, or increased labor efficiency, MAS is a powerful tool to reduce breeding program costs, as this study indicated, by a cost-efficient MAS application in an apple breeding program—which on average removes greater than 25% of individuals. This decision support tool allows breeders to conduct break-even point and sensitivity analyses before implementing any new technologies, providing insight into internal cost savings.

Cost efficiency of MAS in apple breeding is partially enhanced by the prolonged juvenile phase of tree crops, which accrues high costs for labor. Previous studies identified the early juvenile phase (greenhouse stage) as the optimal timing for MAS applications in tree fruit breeding (Edge-Garza et al., 2015; Luby and Shaw, 2001). Early testing allows removal of inferior individuals before they reach seedling test orchards, a method that results in increased cost per individual. This study identified the break-even point where additional costs incurred from MAS integration equals the cost savings for seedling maintenance and evaluation. Our findings suggest that MAS is cost efficient for the apple breeding program if implementation results in removal rates greater than 11.93%. Removal rates greater than this threshold could result in reduced maintenance and evaluation costs, increasing internal economic efficiency for MAS. If removal rates fall below this threshold, MAS is no longer cost efficient. Removal rate has been identi-

fied in previous studies to have a larger impact on MAS cost efficiency than the cost of MAS technology itself (Edge-Garza et al., 2015). The removal rate for the UMN apple breeding program in recent years is ≈50%, which indicates that MAS use in the program is cost efficient.

As new DNA-informed breeding techniques arise, further opportunities for unique program cost structures simultaneously arise. The possibility of drastically reducing specific stages based on advanced techniques (such as the reduction of seedling test orchards to a couple years, based on genomic selection or more aggressive MAS) becomes a theoretical possibility. However, complete elimination of a stage is currently not a possibility because *subsets* of seedlings generated each year undergo marker testing—not the entire seedling population. Additionally, MAS is a valuable tool aiding in improved breeding efforts; but generation of new cultivars may not be wholly reliant upon such technology, because novel traits may arise and require further evaluation.

Removal rate contributes significantly to MAS cost efficiency, but it is not the only factor that should be considered when examining MAS in a breeding program. Marker technology costs still prohibit mass adoption of MAS (Luby and Shaw, 2001; Ru et al., 2015). The sensitivity analyses examined reduced laboratory costs for MAS procedures and indicate that MAS technologies have potential for greater cost savings as the technology costs decrease. Lower costs will allow integration of marker technology in programs that tradi-

tionally could not afford MAS implementation due to low removal rates from marker information. The spreadsheet tool used for this analysis can inform breeders that, when appropriate markers are available, MAS is an effective tool for apple breeding.

Labor costs constitute a significant portion of program costs for the program modeled and other breeding programs (Dreher et al., 2003). The second and third sensitivity analyses were conducted to estimate the impacts of theoretical cost reductions to maintenance and evaluation methods, respectively. These sensitivity analyses show that as labor cost decreases (i.e., through automation) for conventional methods, the total cost remains about $230,000 more expensive than MAS with a 50% removal rate. Our results indicate that with reduced labor costs, conventional apple breeding methods costs remain greater than the cost savings provided by current MAS removal rates for the program.

After removal, breeders may maintain a constant number of individuals entering seedling test orchards on the basis that the maintenance program has already demonstrated the labor capacity to handle that number of individuals (5000 to 6000 in the UMN breeding program). The number of individuals screened at the greenhouse stage may be increased to maintain the number for seedling test orchards. The constant number of individuals entering seedling test orchards would result in increased costs above the break-even point threshold, because MAS costs would increase costs without reducing field labor. However, increased processing of seedlings could generate more cultivars through enrichment of the selection pool in the seedling test orchard. Due to uncertainties in performance of new cultivars in the marketplace, these benefits are difficult to estimate from a financial perspective; and thus our current method shows the potential financial benefits of MAS most readily.

The apple program modeled is similar with other fruit tree breeding programs, although each program will have nuances in its procedures. The flexibility of this tool attempts to account for these nuances, to allow a range of programs (beyond apple breeding) the opportunity to examine their procedures and expenditures. Currently the spreadsheet-based tool provides decision support for comparison of cost efficiency between current methods of breeding with alternative or improved methods. This study used the tool to focus on quantifying MAS cost efficiency in an apple breeding program, and our work provides modeling and simulation that supported previous claims that MAS is a powerful breeding tool capable of reducing breeding program costs (Dreher et al., 2003; Edge-Garza et al., 2015; Slater et al., 2013).

The tool used in this study to estimate MAS impacts on the apple breeding program is not limited to apples nor to the examination of marker technologies. Rather, the tool captures the entire process of breeding a new cultivar. It allows examination of the effects of varying of any cost or breeding information

within the program based on breeder inputs. The detailed input allows flexibility beyond that available in previous decision support tools. This flexibility allows for estimating effects of new management practices, high throughput phenotyping, additional procedures, or any number of variations a breeder might evaluate for implementation in a program. Further studies using the decision support tool in different horticultural crops will elucidate the tool's reliability and usefulness to accurately represent varied breeding program procedures.

Literature Cited

Afanador, L.K., S.D. Haley, and J.D. Kelly. 1993. Adoption of a "mini prep" DNA extraction method for RAPD marker analysis in common bean (*Phaseolus vulgaris* L.). Annu. Rep. Bean Improv. Coop. 36:10–11.

Alpuerto, V.L.E.B., G.W. Norton, J. Alwang, and A.M. Ismail. 2009. Economic impact analysis of marker-assisted breeding for tolerance to salinity and phosphorous deficiency in rice. Rev. Agr. Econ. 31(4):779–792.

Baumgartner, I.O., A. Patocchi, J.E. Frey, A. Peil, and M. Kellerhals. 2015. Breeding elite lines of apple carrying pyramided homozygous resistance genes against apple scab and resistance against powdery mildew and fire blight. Plant Mol. Biol. Rpt. 33(5):1573–1583.

Collard, B.C. and D.J. Mackill. 2008. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363(1491):557–572.

Dreher, K., M. Khairallah, J.M. Ribaut, and M. Morris. 2003. Money matters (I): Costs of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. Mol. Breed. 11(3):221–234.

Edge-Garza, D.A. and C.P. Peace. 2009. July. Enabling marker-assisted seedling selection in the Washington apple breeding program, p. 369–373. In: International Symposium on Molecular Markers in Horticulture 859.

Edge-Garza, D.A., J.J. Luby, and C. Peace. 2015. Decision support for cost-efficient and logistically feasible marker-assisted seedling selection in fruit breeding. Mol. Breed. 35(12):1–15.

Edge-Garza, D.A., N.C. Oraguzie, J. Olmstead, C.P. Peace, A. Iezzoni, S. Haldar, and S. Haendiges. 2009. July. Applying genetic markers for self-compatibility in the WSU sweet cherry breeding program, p. 375–380. In: International Symposium on Molecular Markers in Horticulture 859.

Evans, K., Y. Guan, J. Luby, M. Clark, C. Schmitz, S. Brown, B. Orcheski, C. Peace, E. Van De Weg, and A. Iezzoni. 2012, May. Large-scale standardized phenotyping of apple in RosBREED. In: IV International Conference Postharvest Unlimited 2011 945:233–238.

Evans, K. and C. Peace. 2017. Advances in marker-assisted breeding of apples, p. 189–216. In: K. Evans (ed.). Achieving sustainable cultivation of apples. Burleigh Dodds Science Publishing, Cambridge, UK.

Frey, J.E., B. Frey, C. Sauer, and M. Kellerhals. 2004. Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. Plant Breed. 123(6):554–557.

Gallardo, R.K., H. Li, C. Yue, J. Luby, J.R. McFerson, and V. McCracken. 2015. Market intermediaries' ratings of importance for rosa-

ceous fruits' quality attributes. Intl. Food Agribus. Mgt. Rev. 18(4):121–154.

Hancock, J.F. 2008. Temperate fruit crop breeding: Germplasm to genomics. Springer Science & Business Media, Berlin, Germany.

Iezzoni, A., C. Weebadde, J. Luby, C. Yue, E. van de Weg, G. Fazio, D. Main, C.P. Peace, N.V. Bassil, and J. McFerson. 2010. RosBREED: enabling marker-assisted breeding in Rosaceae. In: International Symposium on Molecular Markers in Horticulture 859:389–394.

Janick, J. and J.N. Moore (eds.). 1996. Fruit breeding: Vol. 1. Tree and tropical fruits. Wiley, Hoboken, NJ.

Johnson, R. 2003. Marker-assisted selection. Plant Breeding Reviews: Part 1: Long-Term Selection: Maize 24:293–309.

Kellerhals, M., M. Spuhler, B. Duffy, A. Patocchi, and J.E. Frey. 2007. Sept. Selection efficiency in apple breeding, p. 177–184. In: XII EUCARPIA Symposium on Fruit Breeding and Genetics 814.

Kenis, K., J. Keulemans, and M.W. Davey. 2008. Identification and stability of QTLs for fruit quality traits in apple. Tree Genet. Genomes 4(4):647–661.

Kim, M.Y., K.J. Song, J.H. Hwang, Y.U. Shin, and H.J. Lee. 2003. Development of RAPD and SCAR markers linked to the *Co gene* conferring columnar growth habit in apple (*Malus pumila* Mill.). J. Hort. Sci. Biotechnol. 78(4):512–517.

Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci. 38(5):1164–1174.

Kuchel, H., G. Ye, R. Fox, and S. Jefferies. 2005. Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy. Mol. Breed. 16(1):67–78.

Kumar, S., D. Chagné, M.C. Bink, R.K. Volz, C. Whitworth, and C. Carlisle. 2012. Genomic selection for fruit quality traits in apple (*Malus ×domestica* Borkh.). PLoS One 7(5):e36674.

Lande, R. and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124(3):743–756.

Liebhard, R., B. Koller, L. Gianfranceschi, and C. Gessler. 2003. Creating a saturated reference map for the apple (*Malus ×domestica* Borkh.) genome. Theor. Appl. Genet. 106(8):1497–1508.

Luby, J.J. and D.V. Shaw. 2001. Does marker-assisted selection make dollars and sense in a fruit breeding program? HortScience 36:872–879.

Moreau, L., A. Charcosset, and A. Gallais. 1998. Marker-assisted selection efficiency in populations of finite size. Genetics 148(3):1353–1365.

Morimoto, T. and K. Banno. 2015. Genetic and physical mapping of *Co*, a gene controlling the columnar trait of apple. Tree Genet. Genomes 11(1):807.

Morris, M., K. Dreher, J.M. Ribaut, and M. Khairallah. 2003. Money matters (II): Costs of maize inbred line conversion schemes at CIMMYT using conventional and marker-assisted selection. Mol. Breed. 11(3):235–247.

Peace, C.P. 2017. DNA-informed breeding of rosaceous crops: Promises, progress and prospects. Hort. Res. 4:17006.

Rudi, N., G.W. Norton, J. Alwang, and G. Asumugha. 2010. Economic impact analysis of marker-assisted breeding for resistance to pests and post-harvest deterioration in cassava. Afr. J. Agr. Resour. Econ. 4:110–122.

Ru, S., C. Hardner, P.A. Carter, K. Evans, D. Main, and C. Peace. 2016. Modeling of genetic gain

for single traits from marker-assisted seedling selection in clonally propagated crops. Hort. Res. 3:16015.

Ru, S., D. Main, K. Evans, and C. Peace. 2015. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet. Genomes 11(1):1–12.

Schneider, K.A., M.E. Brothers, and J.D. Kelly. 1997. Marker-assisted selection to improve drought resistance in common bean. Crop Sci. 37(1):51–60.

Slater, A.T., N.O. Cogan, and J.W. Forster. 2013. Cost analysis of the application of marker-assisted selection in potato breeding. Mol. Breed. 32(2):299–310.

Sorkheh, K., M.K. Dehkordi, S. Ercisli, A. Hegedus, and J. Halász. 2017. Comparison of traditional and new generation DNA markers declares high genetic diversity and differentiated population structure of wild almond species. Sci. Rep. 7(1):5966.

Stromberg, L.D., J.W. Dudley, and G.K. Rufener. 1994. Comparing conventional early generation selection with molecular marker assisted selection in maize. Crop Sci. 34(5):1221–1225.

Suh, J.P., J.U. Jeung, T.H. Noh, Y.C. Cho, S.H. Park, H.S. Park, M.S. Shin, C.K. Kim, and K.K. Jena. 2013. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. Rice 6(1):5.

Tartarini, S. and S. Sansavini. 2002. Aug. The use of molecular markers in pome fruit breeding, p. 129–140. In: XXVI International Horticultural Congress: Genetics and Breeding of Tree Fruits and Nuts 622.

Tartarini, S., S. Sansavini, B. Vinatzer, F. Gennari, and C. Domizi. 1999, Sept. Efficiency of marker assisted selection (MAS) for the *Vf* scab resistance gene, p. 549–552. In: Eucarpia Symposium on Fruit Breeding and Genetics 538.

Testolin, R. 2002, Aug. Marker-assisted selection in stone fruits, p. 163–176. In: XXVI International Horticultural Congress: Genetics and Breeding of Tree Fruits and Nuts 622.

Urbanietz, A., H. Schmidt, and F. Dunemann. 1996. Sept. Molecular markers in early seedling tests for scab and mildew in apples, p. 429–434. In: Eucarpia Symposium on Fruit Breeding and Genetics 484.

Xie, C. and X.U. Shizhong. 1998. Efficiency of multistage marker-assisted selection in the improvement of multiple quantitative traits. Heredity 80(4):489–498.

Xu, K., A. Wang, and S. Brown. 2012. Genetic characterization of the Ma locus with pH and titratable acidity in apple. Mol. Breed. 30(2):899–912.

Yue, C., R.K. Gallardo, V.A. McCracken, J. Luby, J.R. McFerson, L. Liu, and A. Iezzoni. 2012. Technical and socioeconomic challenges to setting and implementing priorities in North American rosaceous fruit breeding programs. HortScience 47:1320–1327.

Yue, C., R.K. Gallardo, J. Luby, A. Rihn, J.R. McFerson, V. McCracken, D. Bedford, S. Brown, K. Evans, C. Weebadde, and A. Sebolt. 2013. An Investigation of US apple producers' trait prioritization—Evidence from audience surveys. HortScience 48:1378–1384.

Zhang, X.J., L.X. Wang, X.X. Chen, Y.L. Liu, R. Meng, Y.J. Wang, and Z.Y. Zhao. 2014. A and *MdMYB1* allele-specific markers controlling apple (*Malus ×domestica* Borkh.) skin color and suitability for marker-assisted selection. Genet. Mol. Res. 13(4):9103–9114.