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ORIGINAL PAPER

Improving the analysis of low heritability complex traits for enhanced genetic gain in potato

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Abstract
Key message **Best linear unbiased prediction (BLUP), which uses pedigree to estimate breeding values, can result in increased genetic gains for low heritability traits in autotetraploid potato.**

Abstract Conventional potato breeding strategies, based on outcrossing followed by phenotypic recurrent selection over a number of generations, can result in slow but steady improvements of traits with moderate to high heritability. However, faster gains, particularly for low heritability traits, could be made by selection on estimated breeding values (EBVs) calculated using more complete pedigree information in best linear unbiased prediction (BLUP) analysis. One complication in applying BLUP predictions of breeding value to potato breeding programs is the autotetraploid inheritance pattern of this species. Here we have used a large pedigree, dating back to 1908, to estimate heritability for nine key traits for potato breeding, modelling autotetraploid inheritance. We estimate the proportion of double reduction in potatoes from our data, and across traits, to be in the order of 10 %. Estimates of heritability ranged from 0.21 for breeder's visual preference, 0.58 for tuber yield, to 0.83 for plant maturity. Using the accuracies of the EBVs determined by cross generational validation, we model the genetic gain that could be achieved by selection of genotypes for breeding on BLUP EBVs and demonstrate that gains can be greater than in conventional schemes.

Introduction

In recent years, major progress has been made in the use of molecular genetic technologies for the identification of genes to enable the implementation of marker-assisted selection in crop improvement programs. However, many of the traits of interest to plant breeders are quantitative in nature and are controlled by few to a very large number of genes of small effect. In order to develop and identify superior genotypes for improvement in such complex traits, methods that can deal with this genetic architecture are required. Best linear unbiased prediction (BLUP) has been successfully used in livestock breeding for this purpose. BLUP, in fact, assumes an infinitesimal model, i.e. an infinite number of genes of infinitesimal effect (Bulmer 1980; Hill 2010) and provides an opportunity for the improved analysis of quantitative traits, especially those of low heritability, in potato.

Most conventional potato (*Solanum tuberosum* L.) breeding strategies employ phenotypic recurrent selection over a number of generations (Bradshaw and Mackay 1994). Typically, a breeding population is generated

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by conducting controlled pollinations to combine selected parents, and the progeny are then subjected to a progression of selection pressures to reduce the population size, while concurrently increasing the number of plants of each genotype that is evaluated (Bradshaw and Mackay 1994; Jansky 2009). In order to reduce the size of breeding populations, the majority of programs practice early generation visual selection to enable more thorough assessment of fewer clones. Prior to the mid 1980s, most potato breeding programs planted a large number of seedlings and then used intensive selection rates to reduce the number of genotypes to a manageable size. Since then, several studies have concluded that the practice of intense visual selection is ineffective, leading to the elimination of superior as well as inferior genotypes (Anderson and Howard 1981; Brown et al. 1984, 1987, 1988; Tai and Young 1984; Bradshaw and Mackay 1994). Breaking visual impression down into individual traits, such as tuber shape, size, number and eye depth, did not improve the effectiveness of selection (Maris 1988). This is because the expression of important visual traits is strongly influenced by environmental effects (low heritability), and seed tuber weight alone was shown to have a significant effect on a number of characteristics (Maris 1986). Some programs that practice intense phenotypic selection (Haynes et al. 2012) have failed to obtain any improvement in yield over time, despite 150 years of breeding (Jansky 2009).

These problems have led to the use of progeny tests to determine better parental combinations (Bradshaw 2007b). Progeny tests will determine the value of the parent for these traits, without identifying the location or number of genes that regulate the expression of the trait.

In potato, progeny testing has been extended to a range of traits. For example, progeny tests for multiple traits (visual preference, late blight tuber and foliage resistances, white potato cyst nematode resistance and fry colour for processing families) have been used by a Scottish breeding program (Bradshaw et al. 2003; Bradshaw 2007b). This program has recently been assessed after 4 cycles of breeding using progeny tests, and superior clones and parental types were identified with improved yield and disease resistance, although the yield increase was small (Bradshaw et al. 2009).

While phenotypic recurrent selection and progeny testing have demonstrated genetic gains, the breeding cycle can be very long (over 10 years) (Jansky 2009). Further, analysis of progeny means will identify the broad sense heritability of the total parental genetic contribution to the family, while calculation of the narrow sense heritability will identify the additive genetic effect and would provide a real benefit to a breeding program that aims to exploit continuous genetic gain.

Animal breeding programs have greatly benefited from the estimation of breeding values using BLUP to exploit the additive genetic variance. BLUP predictions of breeding value are standard practice in animal breeding (Piepho et al. 2008). Animal programs have benefited from estimating the genetic merit of selection candidates based on phenotypic values of all relatives by use of pedigree information in the analysis. For example, significant gains have been seen in milk yield in Holstein cattle in the USA (Van Vleck et al. 1986). Through the use of the phenotypic values of all relatives, the amount of information is maximised and the most accurate genotypic value will be obtained, and it gives a greater increase in accuracy for low heritability traits.

Although BLUP is commonly practiced in animal breeding, it has not as yet been adopted with similar enthusiasm in plant breeding programs (Piepho et al. 2008; Kerr et al. 2012). BLUP is an advanced biometric technique that permits the analysis of a large data set using mixed models and expected genetic covariances, derived from pedigree, for the calculation of breeding values. BLUP can also consider other terms as fixed effects, such as environment and year (Piepho et al. 2008; Hill 2010). BLUP considers information from all relatives in the analysis, therefore increasing the size of the analysed population and improving the accuracy of analysis, particularly for low heritability traits. It also uses more information from relatives that are more closely related to account for the degree of genotypic similarity. To implement BLUP-based breeding, the heritability values of the target traits are required.

As potato is an autotetraploid, and the inheritance of individual genes is more complex than for diploid animals, the relevant inheritance pattern needs to be considered (Casler and Brummer 2008; Kerr et al. 2012) for BLUP analysis. Autopolyploids are formed when multiple chromosome complements arise from the same species (Stebbins 1947; Otto and Whitton 2000; Soltis et al. 2003; Udall and Wendel 2006; Milbourne et al. 2008). As a consequence, all homologous chromosomes can be paired to form multivalents during meiosis. Subsequently, due to chiasma formation and crossing-over, alleles at target loci from sister chromatids can be delivered to the same gamete, a process known as double reduction (Bradshaw 2007a; Milbourne et al. 2008). This inheritance pattern was also considered to be a reason for the failure to widely adopt BLUP in plant breeding programs, as compared with animal breeding programs, by Kerr et al. (2012), who modified the relationship matrix to allow for this more complex inheritance pattern.

The aim of this study was to set the foundation for applying selection on BLUP estimated breeding values (EBVs) in potato breeding programs by

1. Estimating heritability of nine traits which are widely considered in commercial tetraploid potato breeding programs.
2. Investigate the effect of autotetraploidy and double reduction on both heritability estimates and EBVs, by comparing the results to using a (incorrect) diploid model, calculated using standard software.
3. Predicting response to selection on BLUP EBVs, compared with selection on phenotypic values or progeny means, to demonstrate the extra gain that can be achieved in the BLUP schemes. This is done using both real historical data and theoretical predictions of response, to illustrate the greater predictive power and application for selection.

Heritabilities and BLUP EBVs were estimated using a large data set, very deep pedigree information (back to 1908) and restricted maximum likelihood (REML) analysis.

Materials and methods

Population development and trial descriptions

Breeding populations were developed through controlled pollinations of selected parents to develop fruit. At maturity, the fruit was collected and the seeds extracted and washed. The seeds were then germinated, and seedlings were established in trays and transplanted to pots in the glasshouse, which is known as the G0 or the glasshouse seedling generation. Plants were allowed to grow through to senescence. From each pot (plant), a single seedling tuber was collected for planting in the field. Further tubers were retained in family groups for progeny testing.

Tubers collected from the glasshouse seedlings were field-planted at wide spacing. Each plant in this field seedling or first field generation (G1) crop was genetically unique. At maturity, plants were individually harvested by hand to maintain separation. Tubers from each plant were examined, and superior genotypes were visually selected to advance to the next generation. All of the tubers from these selected plants were collected to maximise the size of the selection plots in the following generation. As the tubers form the propagating unit, each genotype within subsequent trials is clonal, and potential cultivars. Samples of tubers were also collected from the rejected genotypes and were retained in family groups for progeny testing of their cooking performance.

Using both cut and whole tubers derived from single plants of the previous season, selections were planted in single short plots (G2) of up to 30 plants for conventional screening. During growth, these genotypes were assessed for plant maturity and early blight resistance, and at harvest the tubers were assessed for breeder's visual preference (BVP), and a reduced set of genotypes was selected and assessed for cooking performance.

Three populations of potential cultivars were studied for this work. The '07' series was germinated in 2006 and contained 13,414 genotypes in the G1 trial in 2007 and 1,132 genotypes across 57 families in the G2 trial in 2008. The '08' series was germinated in 2007 and contained 13,195 genotypes in the G1 trial in 2008 and 1,137 genotypes across 39 families in the G2 trial in 2009. The '09' series was germinated in 2008 and contained 12,720 genotypes in the G1 trial in 2009 and 952 genotypes across 61 families in the G2 trial in 2010. The series names are related to the year in which the series was first planted in the field or G1 generation. Parents were used across multiple years in 191 out of 314 times in the 157 families, and each G2 trial contained common check cultivars for comparison of performance across years.

Phenotyping methods

Plant maturity

Plant maturity was visually assessed by the inspection of plants towards the end of their life-cycle to determine the longevity of each genotype, in comparison with standard cultivars of known maturity. Early genotypes will complete their life cycle in c. 90 days, mid maturity genotypes in c. 105 days and late maturity genotypes in c. 135 days, under the environmental conditions experienced at Toolangi, Victoria, Australia (37°34′S, 145°30′E, elev. 560 m). As 18 categories of maturity were identified, each genotype was allocated a numerical maturity rating for analysis ranging from 0 (for extremely late) to 17 (for very early).

Early blight resistance

Early blight resistance was also visually assessed by inspection of plants towards the end of their life-cycle to determine the level of foliar early blight infection. This was assessed under natural infection conditions, which occur each year under the environmental conditions experienced at Toolangi. Each genotype was allocated a numerical resistance rating for analysis ranging from 1 (for severe symptoms) to 9 (for absence of symptoms).

BVP

At harvest, tubers were laid on the ground, and each genotype was assessed on the basis of the visual characteristics of the tubers. Favourable attributes included required tuber size, shape, topography and uniformity, desired yield, good tuber numbers per plant and skin finish, as opposed to undesirable characteristics such as tuber deformities, secondary growth, cracking, internal disorders, shooting and chaining. All genotypes were allocated a score for BVP that reflected their performance, ranging from 1 (for very poor) to 9 (for very good).

Cooking performance

Selected genotypes were assessed for their specific gravity (SG) and cooking performance. SG was assessed through comparison of the weight of tubers in air compared with their weight in water. Comparison of these values provided an estimation of tuber density, which reflected starch content. Slices were then taken from tubers and cooked in oil at 180 °C for 2 min, in order to determine the effectiveness of cooking by frying. The resulting potato crisps were then scored on the basis of colour, as rated on a numerical scale from 1 (for very light) to 10 (for very dark). Whole tubers were peeled and boiled until cooked. The cooked tubers were then assessed on the basis of boiled flesh colour, any resultant sloughing of the flesh and any after-cooking darkening (ACD) of the flesh that develops within 24 h. The cooked flesh colour was scored from 1 (for white) to 6 (for very dark yellow). ACD levels were scored on a numerical basis, from 1 (for nil) to 5 (for very dark). Sloughing following boiling was scored on a scale from 1 (for nil) to 5 (for total breakdown of the tuber).

Yield

Yield potential of genotypes was assessed in the G3 comparative replicated trial by the comparison of their production against that of standard cultivars for the relevant market use. Tuber numbers were counted and weighed in a range of size categories to determine total yield, marketable yield and the number of tubers per plant. As yield potential was determined in the G3 trial, these values were calculated on a reduced set of genotypes when compared to G2 trials.

Data analysis

Progeny means

To calculate the progeny means of families for the traits of interest, a one-way ANOVA fitting family was conducted on each set of data using Genstat (VSN International Ltd), fitting the model within year

$$\mathbf{y} = \mathbf{1_n}\mu + \mathbf{Wf} + \mathbf{e}$$

where $\mathbf{y}$ is a vector of phenotypes, which could also be described as the response variable for each genotype with a record for the trait being analysed (dimensions number of trait records × 1), μ is the overall mean, $\mathbf{1_n}$ is a vector of ones (number of trait records × 1), $\mathbf{f}$ is a vector of family effects, $\mathbf{W}$ is a design matrix allocating trait records to families and $\mathbf{e}$ is a vector of random residuals.

Estimates of heritability and BLUP EBVs

In order to utilise information from all relatives, a pedigree file was developed using the Potato Pedigree Database (van Berloo et al. 2007), various relevant publications and in-house pedigree records. The pedigree file covered two generations and genotypes dating back to 1908.

The model

$$\mathbf{y} = \mathbf{1_n}\mu + \mathbf{Xb} + \mathbf{Zu} + \mathbf{e}$$

was fitted to the data for each trait separately, where $\mathbf{y}$ is a vector of phenotypic records for that trait (dimensions number of trait records × 1), μ is the overall mean, $\mathbf{1_n}$ is a (number of trait records × 1) vector of ones, $\mathbf{b}$ is a vector of year effects (3 × 1), $\mathbf{X}$ is a matrix allocating records to year effects (number of trait records × 1), $\mathbf{Z}$ is a matrix allocating records to breeding values (dimension number of trait records × number of genotypes in the pedigree), $\mathbf{u}$ is a (number of genotypes in the pedigree) vector of (random effect) breeding values, distributed $\sim N(0, \mathbf{A}\sigma_{\mathrm{g}}^2)$, σ_{g}^2 is the genetic variance, and $\mathbf{e}$ is a vector of random error terms $\sim N(0, I\sigma_{\mathrm{e}}^2)$, σ_{e}^2 is the error variance.

The approach of Kerr et al. (2012) was used to calculate the numerator relationship matrix ($\mathbf{A}$, dimensions number of individual genotypes in the pedigree × number of genotypes in the pedigree). First, a kinship matrix, $\mathbf{K}$, was derived given that all genotypes in our pedigree are autotetraploid. Following Kerr et al. (2012), elements of this matrix (the lower diagonal is defined first, then the matrix is reflected about the diagonal) are: Both parents unknown

$$k_{ii} = \frac{1+w}{4}, \quad k_{ij} = 0(i < j).$$

One parent known

$$k_{ii} = \frac{5 + 7w + 4k_{pp}(1-w)}{24}, \quad k_{ij} = 0.5(k_{ip})(i < j).$$

Both parents known

$$k_{ii} = \frac{1 + 2w + (1-w)k_{pp} + (1-w)k_{qq} + 3k_{pq}}{6},$$
$$k_{ij} = 0.5(k_{ip} + k_{iq})(i < j).$$

When the off-diagonal elements are computed recursively, and w is the proportion of a parent's gametes that are identical by descent (IBD) due to double reduction, k_{pp} is the diagonal element of the matrix for the first parent p, k_{qq} is the diagonal element of the matrix for the second parent

q, and k_{pq} is the pqth element of $\mathbf{K}$. The numerator relationship matrix, $\mathbf{A}$, was calculated as $\mathbf{4K}$.

ASReml3 (NSW Dept. of Primary Industries and VSN International Ltd) was used to fit the mixed model, estimate variance components and predict EBVs ($\hat{u}$). Then heritabilities were calculated as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$.

In the equations to calculate the kinship matrix as shown above, the value of w, the proportion of parental gametes that are IBD due to double reduction, is required. As this value is unknown, different values of w were tried from 0 to 0.25 to investigate which value, when the above model was fitted, maximised the likelihood of the data given the parameters, for each trait. The resulting EBVs from the optimum autotetraploid analysis were compared with the values derived from a standard diploid analysis (e.g. $\mathbf{A}^{-1}$ derived from Henderson's rules) (Henderson 1984).

Estimation of expected genetic gain

Genetic gain was calculated based on the following equation:

$$\Delta_G = h^2 \times \Delta_S \quad (\Delta_S = \text{differential of selection})$$

$$\text{or } \Delta_G = (h^2 \times V_P \times i)/L$$

(h^2 = heritability, V_p = phenotype variation, i = selection intensity, L = length of breeding cycle).

Cross-generation prediction

Mid-parent values, individual genotype values, progeny means and EBVs were used to compare the predictability of cross-generation prediction. This was conducted for a highly heritable trait (SG) and also for a lower heritability trait (BVP). This analysis was conducted for two breeding populations, the '08' and '09' series.

As a result of the analysis of the G1 and G2 data from the '08' and '09' series, which showed that individual genotypes provide a very low cross-generation prediction for BVP, the '10' series G1 population was subjected to an altered selection regime to determine whether a milder selection practice should be employed. A 10–20 % selection rate was typically practiced in the G1 generation, based solely on BVP. For the '10' series, the typical selection method was conducted, obtaining c. 20 % from our first round of selection. A second round of selection was then conducted on the population, aiming to select another 20 % of genotypes that showed some promise. This was then followed by a third round of selections within a few families to collect genotypes with limited promise. The genotypes in the G2 generation were then selected without reference to their original round of selection, and the results were compared.

Development of a selection tool

Potato breeding uses recurrent selection of superior genotypes to reduce the number of candidate genotypes, after screening for a number of desirable traits. As the calculation of EBVs provides a value for each genotype, these values can be ranked for each genotype for traits that are important for the French fry, crisping and fresh markets, respectively. When the desirable trait had a target value within the current range of phenotypic variation, the genotypes were ranked according to how closely they matched the target value (using a linear penalty from the optimal value). Genotypes were ranked in descending order, and the values were added to provide a total score.

Results

Autotetraploid effect on heritability and EBVs

The likelihood of the observed data arising due to different proportions of double reduction in the parents was obtained from the REML analysis of each trait. For five of the nine traits, a value for w of 0 maximised the likelihood of the data given the value of the parameters (Fig. 1). For two of the traits, a value of 0.25 gave the highest likelihood, while the values were 0.1 for maturity and 0.15 for early blight resistance. Therefore, the analysis did not provide definitive results for the proportion of IBD gametes due to double reduction occurring during meiosis, and the impact of accounting for double reduction is limited at these low values (average over traits of approximately 0.1).

With an assumed value of $w = 0.1$, the heritabilities of the traits were similar when assuming diploid or

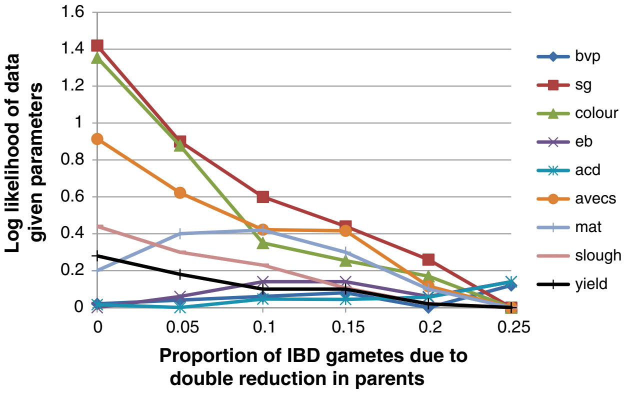

Fig. 1 Maximum log likelihood of the data given the value of the parameters, including the proportion of IBD gametes due to double reduction, for each trait. Log likelihoods are expressed as a difference from the smallest value of the maximum likelihood across the range of values for the proportion of IBD gametes

Table 1 Comparison of the heritability of eight traits when calculated for disomic and tetrasomic inheritance with $w = 0.1$

Trait	Diploid	Autotetraploid with $w = 0.1$
Plant maturity	0.86 ± 0.03	0.83 ± 0.03
Early blight resistance	0.57 ± 0.05	0.44 ± 0.04
BVP	0.23 ± 0.05	0.21 ± 0.05
Total yield	0.56 ± 0.09	0.58 ± 0.09
Tuber SG	0.73 ± 0.04	0.74 ± 0.04
Average crisp score	0.64 ± 0.05	0.59 ± 0.05
Boil colour	0.73 ± 0.04	0.69 ± 0.04
Boil ACD	0.58 ± 0.06	0.54 ± 0.06
Boil sloughing	0.51 ± 0.06	0.48 ± 0.06

autotetraploid inheritance (Table 1). Eight of the nine traits exhibited effectively the same value, while the heritability of early blight resistance was lower when double reduction was considered.

Plant maturity, tuber SG and boil colour showed the highest heritability values, followed by average crisp score, total yield, early blight resistance, ACD and sloughing following boiling, while the lowest heritability value was obtained for BVP.

The heritability estimations were also consistent with expectations when trait expression is considered. BVP heritability is likely to be low, as this trait is actually the composite of many traits of varying heritability and would be expected to be affected by environment and seed size. The early blight resistance ratings would be expected to be affected by seasonal and variable inoculum levels. Total yield would be affected by the growing season conditions. Boil ACD would be affected by environment and population composition. Boil sloughing would be affected by the extent of cooking and population composition. Conversely, tuber SG displayed high heritability, but can be affected by nutritional status. Average crisp score exhibited high heritability, but can be affected by nutrition and cold. Boil colour should show high heritability, as it is not known to be affected by the growing environment. Plant maturity displayed the highest heritability value, but may also be affected by environmental conditions and disease pressure from blight infection. This increase in heritability is likely to also be reflected in the complexity of the genetic control of the trait with BVP comprised of many traits, while maturity has been shown to have quantitative trait loci of major effect (Bradshaw et al. 2004).

Estimation of genetic gain

Genetic gain was not being measured at the start of this study, but following the adoption of progeny testing and

Table 2 Comparison of the expected rate of genetic gain using phenotypic selection of potato traits or BLUP EBVs

Trait	Phenotypic variance (V_p)	Diploid heritability (h^2)	Phenotypic diploid expected genetic gain (units/year)	EBV diploid expected genetic gain (units/year)	Autotetraploid heritability (h^2)	Phenotypic autotetraploid expected genetic gain (units/year)	EBV autotetraploid expected genetic gain (units/year)
Early blight resistance	4.47	0.57	0.373	0.892	0.44	0.288	0.688
BVP	1.77	0.23	0.060	0.143	0.21	0.054	0.130
Total yield	97.59	0.56	7.99	19.13	0.58	8.28	19.81
Boil ACD	0.80	0.58	0.068	0.165	0.54	0.063	0.151

The breeding cycle using phenotypic selection employed a 10 % selection intensity and took 12 years, while the breeding cycle using EBVs employed a 20 % selection intensity and took 4 years. A 10 % selection intensity in standard deviation units under a normal distribution is 1.755, while a 20 % selection intensity is 1.4 (Falconer and Mackay 1996)

Table 3 Progeny means and phenotypic variation for eight traits for the '09' series G2 families

Trait	Scale	Overall mean	SE	Family mean max–min	Individual phenotype max–min
Plant maturity	0–17	8.19	1.38	4.0–11.5	1–15
Early blight resistance	1–9	4.33	1.02	2.0–8.0	1–9
BVP	1–9	4.68	0.67	2.0–6.23	1–8
Tuber SG	1.05–1.11	1.078	0.004	1.063–1.097	1.054–1.107
Average crisp score	1–10	8.00	0.56	4.63–9.74	2.2–10
Boil colour	1–6	3.59	0.47	1.0–4.91	1–5.5
Boil ACD	1–5	2.06	0.50	1.0–4.0	1–5
Boil sloughing	1–5	2.17	0.56	1.0–4.5	1–5

Table 4 Comparison of the '09' series G2 progeny means with the EBVs calculated with and without allowance for double reduction

Trait	Progeny mean and diploid EBV correlation	Progeny mean and autotetraploid EBV correlation	Diploid EBV and autotetraploid EBV correlation
Plant maturity	0.69	0.74	0.97
Early blight resistance	0.59	0.71	0.89
BVP	0.59	0.61	0.96
Tuber SG	0.92	0.93	0.99
Average crisp score	0.72	0.73	0.98
Boil colour	0.86	0.86	1.00
Boil ACD	0.76	0.78	0.99
Boil sloughing	0.82	0.82	1.00

calculation of EBVs, it could be predicted and measured. The Australian breeding program has historically used a selection intensity of 10 %, with a breeding cycle of c. 12 years, which would provide an estimated genetic gain for total yield of c. 8 tonnes per hectare per year (Table 2). Through the use of EBVs, these predicted values could be significantly increased to c. 19 tonnes per hectare per year (Table 2), despite the reduction of selection intensity to 20 %, as the breeding cycle is reduced in duration to 4 years as a result of the identification of genotypes with superior breeding values.

Comparison of progeny means and EBVs

The overall mean of the '09' series for the eight traits, as well as the maximum and minimum values of phenotypic variation, is shown in Table 3. The progeny means of the '09' series families for each of the traits are listed in Supplementary Table 1. The mean values for the families were ranked, allowing the identification of the superior families for each trait. Interestingly, when they were ranked for BVP, four of the five lowest ranked families did not exhibit any progeny suitable for collection, and their cooking performance was consequently not assessed.

A comparison of the '09' series family progeny means for BVP with their corresponding diploid and autotetraploid EBVs revealed a high degree of similarity, although differences were observed between the progeny means and the EBV values when the families were ranked for their respective values (Supplementary Table 2).

The relationship between both sets of EBVs and the progeny means varied, although all displayed a positive relationship. For all traits (apart from early blight resistance), the relationship between the progeny means and either the standard diploid EBVs or the autotetraploid EBVs were similar, as reflected in the very strong correlation between the EBVs of over 0.95 for 8 of the 9 traits (Table 4).

Cross-generation prediction

As potato breeding uses recurrent selection across a series of generations and screening trials to identify superior cultivars, it is important to understand the reliability of parental and early generation phenotypes for the prediction of each genotype's final phenotype. Mid-parent values, progeny tests and EBVs can be used to determine the optimal predictive model for the determination of reliable expression in subsequent generations. This comparison was performed

Table 5 Correlation between mid-parent and cross generation values for SG and BVP

Comparison	'09' series SG	'08' series SG	'09' series BVP	'08' series BVP
G2 versus G1 individual genotype comparison	–	–	0.18	0.21
G2 family means versus mid-parent value	0.87	0.87	0.02	0.14
G2 family means versus G0 family means	0.59	0.81	–	–
G2 family means versus G1 family means	0.78	0.77	0.37	0.42
G2 family diploid EBVs versus mid-parent value	0.94	0.88	–	–
G2 family autotetraploid EBVs versus mid-parent value	0.93	0.89	–	–
G2 family diploid EBV versus G1 family diploid EBV	–	–	0.41	0.53
G2 family autotetraploid EBV versus G1 family autotetraploid EBV	–	–	0.34	0.53
G2 family means versus mid-parent diploid EBV	0.92	0.95	0.59	0.74
G2 family means versus mid-parent autotetraploid EBV	0.93	0.97	0.58	0.74
G2 family diploid EBV versus mid-parent diploid EBV	1.00	0.99	1.00	0.93
G2 family autotetraploid EBV versus mid-parent autotetraploid EBV	1.00	1.00	0.96	0.94

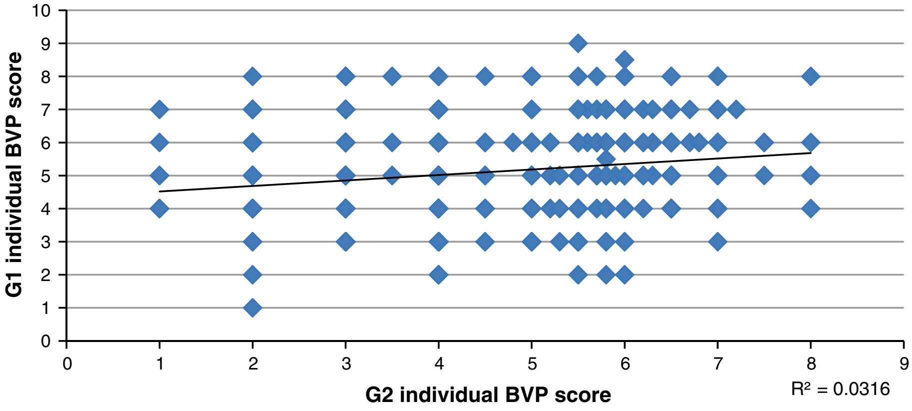

Fig. 2 Comparison of the '09' series G1 and G2 BVP scores for individual genotypes

for a highly heritable trait (SG) and an important trait with low heritability (BVP).

Collection of data to calculate progeny means and EBVs permits comparison between genotypes and families across successive field generations, to determine the optimum selection strategy.

SG: a high heritability trait

Specific gravity is highly heritable (0.74, Table 1) and was found to display good correlation across generations, including the glasshouse and field seedling generations, although these exhibited the weakest relationship with the G2 family means. A good relationship was displayed between the mid-parent values and the G2 family means, which was improved when the mid-parent EBVs were used. The mid-parent diploid and autotetraploid EBVs showed a strong correlation (>0.92), when compared with the G2 family means (Table 5). These improved relationships were consistent for both the '08' and '09' series (Table 5).

Table 6 Comparison of the selection pressure on the '10' series G1 population to the G2 individuals selected

	% G1 selected	% of G2 trial	% selected from G2
Bag 1	25.7	46.2	57.4
Bag 2	22.5	40.4	34.0
Bag 3	7.4	13.4	8.7
Total	55.5	100	

BVP: a low heritability trait

BVP has a low heritability (0.21), as shown in Table 1, reflecting the concurrent expression of a number of traits, and is likely to be affected by both growing environment and seed size. When G1 and G2 BVP scores were compared for individual genotypes across two breeding populations (the '09' and '08' series), the correlation was low, but positive (Table 5). Both populations contained high- and low-scoring genotypes in both generations. However, other genotypes scored low in the G1 and high in the G2, while

Table 7 '09' series top 10 genotypes ranked by EBVs for French fry characteristics of BVP, SG and average crisp score

Cultivar	BVP	SG	Ave Crisp	Total	Rank	French fry comment
09-02-02	944	947	875	2,766	1	Not enough for G3, back with G2
09-60-20	916	896	829	2,641	3	French fry G3 trial
09-60-14	943	945	725	2,613	4	French fry G3 trial
09-02-01	956	836	773	2,565	6	French fry G3 trial
09-53-05	879	908	771	2,558	7	French fry G3 trial
09-60-06	812	861	877	2,550	9	Not enough for G3, back with G2
09-52-01	981	895	669	2,545	10	French fry G3 trial
09-60-01	902	873	760	2,535	11	French fry G3 trial
09-60-12	871	823	822	2,516	14	French fry G3 trial
09-53-01	879	894	739	2,512	15	French fry G3 trial

others showed the converse relationship (Fig. 2). There was also a poor relationship when the mid-parent values were compared with the mean G2 family means. This outcome indicated that neither the mid-parent values nor early generation scores were reliable.

The relationship was improved when family means were compared across the same two data sets, and further gains were made when the mid-parent diploid or autotetraploid EBVs are used (Table 5). The mid-parent EBVs provide a significant improvement over the mid-parent value as it predicts the G2 family means with an accuracy of 0.59 (0.74 for '08') compared with 0.02 (0.14 for '08') for the mid-parent value (Table 5).

Modified BVP selection rates for the '10' series

As there was a very limited relationship between the G1 and G2 individual genotypes in the '08' and '09' series (Table 5), the selection rate was altered in the field for the '10' series to determine if superior G2 genotypes would have been rejected at the G1 stage. The modified selection practice on the '10' series shows that 25 % of the G1 population was selected in the first round, an additional 23 % in the second round and a further 7 % in the third round, providing a selection rate of over 50 % from the G1 population (Table 6). The G2 generation selection showed that the majority of chosen genotypes were from the first G1 selection round, as expected, but a substantial proportion (34 %) was also from the second round and an appreciable number (9 %) of genotypes were even obtained from the third round of selections (Table 6). These results indicate that a very mild selection pressure should be placed on the breeding populations if based on BVP alone, which is the case for the G1 generation. When a more intense selection pressure is used, a significant number of genotypes that could have been selected in the G2 generation would have already been rejected at the G1 generation stage.

Using EBVs as a selection tool to advance superior cultivars

After the phenotypic G2 data from the '09' series had been analysed and the individual cultivars had been identified to advance to the G3 trial, progeny EBVs were ranked for the most important characters to retrospectively determine whether EBVs could have been used to determine which cultivars should advance. The progeny EBVs were modelled for the relevant markets, and the top ranked individual genotypes were identified in each sector. As an example, the top 10 French fry genotypes are listed in Table 7, after their EBVs were ranked and the rankings were combined for a total score specific to the French fry characteristics of BVP, SG and average crisp score. The higher ranking genotypes should then be assessed for other important traits before inclusion in further trials. All of the top genotypes retrospectively ranked for their combined EBVs had been either placed into the G3 trial, or returned for further assessment with the G2 genotypes, due to lack of propagating material.

Discussion

Estimates of the heritability for nine important traits in a potato breeding program were obtained by modelling autotetraploid inheritance. Our analyses did not give a consistent estimate of the proportion of double reduction in potatoes from our pedigree and phenotype data across traits, but the results suggest that this value is small, in the order of 10 %. This study has demonstrated the potential benefits of using BLUP EBVs in potato breeding programs, clearly demonstrating the advantage of using EBVs over progeny means in cross-generation prediction of progeny performance, particularly for traits with low heritability.

Recently, progeny means have been used to enable simultaneous selection for quantitative disease resistance, breeder's preference and fry processing colour, leading to improvements in most, but not all, traits. No progress was seen in visual preference, and only a moderate improvement was seen in yield (Bradshaw et al. 2009).

As the BLUP analysis uses the genetic information from all relatives, including full-sibs, half-sibs and any other partial siblings, the estimation of the breeding value should be improved, with greater accuracy than when only using information from the full-sibs, as occurs in the progeny mean analysis. However, both progeny means and BLUP are more accurate than phenotypic selection for low heritability traits, which are affected by environmental factors. By using the information from all relatives the BLUP analysis also enabled the analysis of a much smaller population in the third field generation for the important trait of total yield. EBVs also estimate only the additive genetic effect (Falconer and Mackay 1996), which is the component that is transmitted from parents to progeny. While ANOVA can calculate mean values for each of the families, BLUP analysis provided EBVs for the families, and all cultivars in the pedigree and the individual genotypes themselves, potentially enabling breeding from the best progeny.

Calculation of progeny means over simple use of phenotypic identification of individuals improves the analysis, as it identifies the total genetic content of the parents, while EBV identification should enable more rapid genetic gain in potato breeding programs, particularly for moderate to low heritability traits such as yield. Our modelling suggests that by using EBVs, the expected genetic gain for each trait is predicted to more than double as a result of this study.

EBVs are commonly used in animal breeding although they have not been as widely adopted in plant breeding (Piepho et al. 2008; Kerr et al. 2012). A number of reasons have been postulated for this lack of adoption, including the more complex inheritance patterns displayed by a number of crop plants. As potato is an autotetraploid crop, Kerr et al. (2012) recommended modification of the numerator relationship in the BLUP analysis to account for such inheritance patterns. An effect was found when the results of the assumed diploid analysis were compared with the modified autotetraploid analysis, although a very strong relationship between EBVs calculated under the two assumptions was obtained (>0.95) for 8 out of the 9 traits. From this it can be concluded that, while consideration of the autotetraploid inheritance pattern will provide a more accurate EBV, use of the more easily derived **A** matrix under diploid inheritance in the BLUP analysis would have also provided reasonable values and should not have prevented its adoption. Performance of the autotetraploid analysis also required an investigation of the average rate of double reduction, or the number of gametes that are IBD for the genetic loci controlling these traits, which was 0 % for 5 traits, 10 and 15 % for 1 trait each and 25 % for 2 traits, providing an overall average close to 10 %. The 25 % rate is consistent with the prior prediction of 1 in 4 (Luo et al. 2006), although the 0 or 10 % rate is much lower, and could represent a mixture of bivalent and multivalent pairing across the loci.

The EBVs were also observed to improve cross-generational prediction. As potato breeding programs endeavour to progressively reduce the size of the breeding population, effective prediction of the genotypic performance in latter generations is important to ensure that superior genotypes are retained while only maintaining a population that is not too large or expensive to evaluate. For both the highly heritable SG trait and the lowly heritable BVP trait, mid-parental EBVs displayed the closest relationship with the actual phenotypic means of the G2 families. While an improvement was observed for the highly heritable trait, there were more substantial improvements made for the lowly heritable trait, showing that EBVs should provide a more reliable prediction method for traits with low heritability.

The unreliability of individual genotypic performance from the first field generation to the second was again seen in the actual breeding program, based on phenotypic selection across three breeding populations, culminating with a selection of significant number of genotypes in the second generation that would have been rejected from the first field generation of the '10' series. This outcome is consistent with the outcomes of a number of other studies (Anderson and Howard 1981; Brown et al. 1984, 1987, 1988; Tai and Young 1984; Bradshaw and Mackay 1994) and has led to an alteration in the selection rate in the first field generation of the Australian program, from a moderate 10 % to a much higher selection rate of c. 30 %. These results are also in accordance with the selection strategies for a number of other crops, as described by Simmonds (1985).

Due to the unreliability of intense selection, studies have also been undertaken to look at alternative methods to reduce population numbers without loss of superior genotypes. Mid-parent values and mean progeny analysis have been used to discard whole progeny sets prior to selection within progenies (Bradshaw et al. 2003, 2009; Diniz et al. 2006; Melo et al. 2011). However, these methods could still potentially lead to the loss of superior genotypes that are located within the average or lower valued families, although they would be present in lower numbers than in the higher scoring families (Diniz et al. 2006). As the BLUP analysis can provide an EBV for individual genotypes, these superior genotypes will be identified even if located in the average or lower scoring families. BLUP analysis will, therefore, identify superior genotypes for retention that would otherwise have been lost by the rejection of entire lower scoring families.

The BLUP analysis provides a number of advantages over current selection methods used in potato breeding. As an added advantage, calculation of EBVs for a number of important traits has permitted a combined ranking of evaluated genotypes or the development of selection indices across multiple traits (Xu et al. 2012). This process should enable identification of genotypes based on properties of the population in addition to individual phenotypes and provide a more accurate selection process for these traits. As well as those traits assessed in this study, BLUP could be applied to any desirable quantitative character, including those of very low heritability.

Genetic improvements in livestock breeding over the past 30 years have led to significant growth in a number of industries, particularly in poultry and pigs, but also in dairy cattle (Hill 2010; Thornton 2010). These improvements have been attributed to a number of factors, not least the more efficient statistical methods for the estimation of genetic value of individuals using BLUP prediction (Hill 2010; Thornton 2010). The adoption of BLUP EBVs for the rapid identification of superior families and individuals could see similar genetic improvements in potato, as well as other crops.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All experiments performed comply with the current laws of Australia.

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