



Is plant variety registration keeping pace with speed breeding techniques?

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Abstract Recent innovations in breeding technologies have reduced the timeframe to develop improved plant varieties compared to conventional breeding processes. Technologies like speed breeding, or rapid generation advancement, may also accelerate the process of statutory variety registration. Within this procedure, improved varieties are required to satisfy distinctness, uniformity and stability (DUS) criteria to establish the unique identity of a given submission during the variety registration process. The DUS standard also provides a solid basis for seed certification, plant breeders' rights, as well as variety maintenance throughout commercial lifespan of varieties. Currently, the overall timeline of variety registration may vary from 2 to 4 years, depending on crop type and country. In this article, we propose the concept of 'speed DUS testing': a rapid phenotype-based method, which could be integrated with approaches that take advantage of DNA markers. We compare methods and

discuss how DUS testing could be modernized to fast-track variety registration.

Keywords Plant variety registration · Speed breeding · DUS testing · DNA markers

Abbreviations

DUS Distinctness, uniformity and stability
PBR Plant breeder's rights
VCU Value for cultivation and use

Why and how plant varieties are registered

It is very important for farmers to know which variety they buy and grow. The variety determines, to a large extent, the success of a crop in terms of adaptation to the local agro-ecology and the value of the resulting produce in the market. Farmers who buy seed depend on reliable information about the variety, which was initially provided by the seller. For instance, the posters developed by Vilmorin in France during the mid-nineteenth century depicted the types of vegetables that they sold. With the development and more widespread application of plant breeding from the nineteenth century onwards, more distinct varieties entered the market, which in turn led to calls for independent information to be generated, in addition to the catalogues provided by seed companies. This

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resulted in lists of recommended varieties based on agronomic data, and official variety registration in national variety lists determining varietal identity that create transparency in the market. Thus, as an initial aim, variety registration allowed a variety to be uniquely identified with its allocated name and so relieve farmer's confusion with respect to varietal identity. This was important because varieties were often marketed under a popular name and different seed merchants used different names for the same variety (Louwaars and Burgaud 2016). In addition, such varietal identity provides a solid base for seed certification, plant breeder's rights (PBR), and variety maintenance throughout its commercial lifespan.

Currently, in many countries, registration is based on a statutory mandate before releasing a variety, as it links variety development to seed certification and marketing (Van Wijk and Louwaars 2014). The scope of species for registration varies between countries, with the most comprehensive being in the European Union (EU) which covers almost all varieties of agricultural crops, vegetables and fruit species¹ compared to, for instance, subjectivity of only certain major field crops such as cereals, canola, and flax in Canada.²

To be eligible for registration, new plant varieties should be 'Distinct' from all previously registered varieties in at least one morphological trait (to satisfy a unique identity) and have sufficient 'Uniformity' and 'Stability' in expression of their inherent traits (Jamali et al. 2019). Collectively, the distinctness, uniformity and stability (DUS) criteria are assessed in the field or glasshouse over two or more independent growing cycles, dependent on the crop. These technical standards are also employed in plant variety protection (PVP) systems through provisions modelled by the international union for protection of new varieties of plants (UPOV) convention. DUS criteria can play interchangeable purposes in registering a variety (approval for marketing) as well as granting PBR to variety owners. However, distinction (D within DUS) of candidate varieties for protection may be fulfilled

through comparison with common knowledge varieties, while for listing purposes (variety registration) a panel of already registered varieties in national lists would be sufficient for examination (van Wijk and Louwaars 2014).

Additionally, the pre-market variety registration procedure mandates that crop varieties have equal or superior 'value for cultivation and use' (VCU) over the standard ("check") varieties (Cooke and Reeves 2003). In that respect, specific adaptation to different agro-ecological conditions is outweighed by wide-range adaptability of varieties across multiple environments, as decided by most variety release committees. Especially in countries where there is no requirement for registration of all plants before variety release (e.g. in Australia), or where certain crops are excluded (e.g. maize, food-grade soybean, chickpea and turf grasses in Canada), performance trials are integral parts of breeding programs whereby yield, disease resistance and quality traits of superior promising lines are tested across multiple environments (Louwaars and Burgaud 2016). In such instances, DUS testing merely plays the role of providing a 'variety description' of protected varieties that could be subsequently used within the seed certification process (Fig. 1). PBR bestows upon the variety owner the legal ability to prevent others from commercial use without consent (although exploitation of protected germplasm for research or breeding further varieties is exempted from PBR scope). Furthermore, variety description allows breeders to identify their varieties in case of PBR infringement. It is especially crucial for autogamous and vegetatively-propagated species whose seeds and propagating materials could be easily multiplied by others, hence sidestepping lengthy and costly breeding programs. In addition, protection of varieties is a well-suited measure for funding further research and development activities through the collection of royalties (Sanderson and Adams 2008). A good example is establishment of the end point royalty collection system in Australia for crops like wheat and barley, where a fee (generally up to 5 Australian Dollars per tonne of production) is paid by the grower to the PBR holder depending on how much is harvested from a protected variety at the end of the season.

Irrespective of the systems employed, from official (in most EU countries) to breeder testing (in Canada) (Van Wijk and Louwaars 2014), current DUS testing

¹ European Directive 70/457/EC (1970), revised in 2002 (2002/53/EC).

² Crop Variety Registration in Canada, Issues and Options, the Minister of Agriculture and Agri-Food, 2013, http://canada.usgrainandseedtrade.info/wp-content/uploads/2014/01/vr-ev_option-en.pdf.

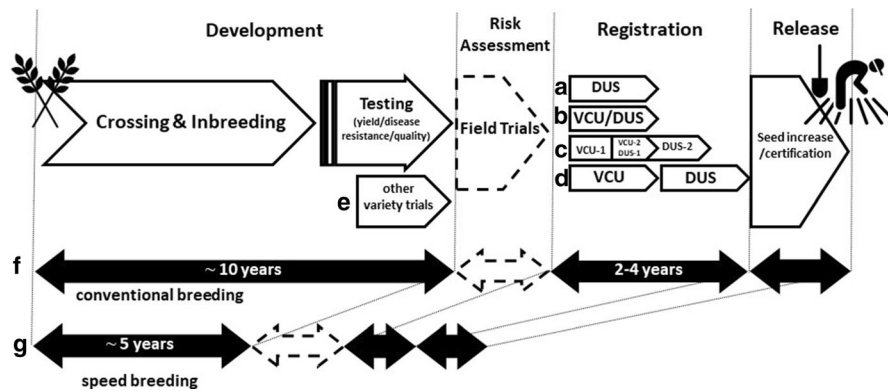


Fig. 1 Schematic time frame for variety development, risk assessment, registration and release of an inbreeding crop species. In countries with compulsory variety registration, DUS may be solely regarded sufficient for registration of e.g. sunflowers in Canada (a), may be conducted simultaneously with VCU trials (b), may be lagged for 1 year upon feedback from VCU data (c), or be carried out after completion of VCU trials (d). These four scenarios mean registration may be completed within a 2–4 year timeframe. It is also possible that performance testing step (of variety development) or field trials of risk assessment (dashed shapes) relating to transgenic or gene-edited crops (only in EU based on Court of Justice ruling of 2018) be accepted as equivalent as VCU data. Here, crop

varieties may pass through DUS step (a) to be registered. This scenario may be employed in countries with no requirement for registration for all (e.g. Australia) or certain species (e.g. Canada), where VCU/merit assessment is conducted in parallel to latter steps of breeding program (e). But here, DUS testing provides varietal identity for immediate seed certification process. It is expected that implementing either of options i.e. ‘speed DUS testing’, ‘diagnostic markers’, ‘combined phenotypic and genetic distances’, and ‘varietal description by DNA sequencing’ associated with DNA-based seed certification and ‘speed breeding’ methods (g) will reduce the number of years needed to release new plant varieties, compared to conventional approaches (f)

generally relies on morphological (phenotypic) characteristics (hereafter termed DUS traits) that are in theory not, or minimally, affected by environmental factors and genotype-by-environment interactions. The tests are conducted according to species-specific guidelines provided by UPOV.³ The process of establishing these guidelines involves proposal by UPOV, followed by discussion and agreement of the member states. Breeders are also invited by UPOV and most of the individual member states to comment on the guidance. DUS traits may be qualitative (e.g. the barley character ‘hairiness of leaf sheaths of lowest leaves’), pseudo-qualitative (e.g. barley ‘colour of aleurone layer of kernel’) or quantitative (e.g. barley ‘plant height’). Moreover, additional characteristics may be derived from electrophoresis of storage proteins (e.g. hordeins in barley) for ‘Distinctness’ assessment (UPOV 2018a). In contrast to DUS testing, there is no international coordination on the methods for conducting VCU trials, and these may vary between countries (Waes 2006).

Potential shortcomings of DUS traits

The initial requirements of phenotype-based DUS criteria for variety registration in Europe (as early as 1920s) was a precursor for implementing them as technical rules in UPOV’s PVP system of 1961 (Louwaars and Burgaud 2016). It is plausible to assume that identification of varieties through phenotypic descriptions in those times was better congruent with the employment of phenotypic selection by plant breeders. Use of DUS criteria have remained intact in all three revisions of the UPOV convention in 1972, 1978, and 1991 (the latest Act) until now. However, while plant breeders have now begun to incrementally diminish their reliance on phenotypes alone for selection of desirable traits, largely through use of DNA markers in marker-assisted selection (MAS) and genomic selection (GS) methods, UPOV has yet to reflect such changes within its existing systems. In addition, the gap would be widened once plant breeders use genomic data e.g. DNA sequence of breeding materials (Varshney et al. 2018) or knowledge-driven combination of desirable haplotypes (Abbai et al. 2019; Sinha et al. 2020) in variety improvement. Thus, the almost exclusive use of

³ http://www.upov.int/test_guidelines/en.

phenotype-based DUS traits has been previously highlighted as a possible weakness of the PVP system (Janis and Smith 2007). However, UPOV does recognize the possibility of a complementary role for DNA markers in DUS test guideline procedures (UPOV 2013), as discussed in more detail in the sections below.

This position could be also explained by the challenges of applying phenotypic criteria in characterizing the ever-increasing number of plant varieties, which annually makes the management of reference collections more costly for DUS examination (Lombard et al. 2000). Side-by-side comparisons of ever-increasing numbers of varieties become laborious and time-consuming (Tian et al. 2015), and the options for distinction via morphological differences between candidates and larger numbers of reference varieties may become increasingly difficult (UPOV 2016). In addition, DUS traits may be a barrier for release of improved varieties. For instance, some records show that a quarter of alfalfa (*Medicago sativa* L. ssp. *sativa*) and one-tenth of *Lolium* spp. and white clover (*Trifolium repens* L.) varieties with sufficient VCU are not registered (and so don't achieve approval for marketing) due to lack of DUS Distinctness from previous varieties (Annicchiarico et al. 2016; Gilliland and Gensollen 2010). To compensate for this, agronomic traits are used as part of the description in some species, e.g. seed number per spikelet and thousand-seed weight for ryegrass (Wang et al. 2016), and sugar content, root yield and sugar yield for sugar beet.⁴ These traits are not capable of precise discrimination between varieties as they have relatively low consistency owing to significant interaction with environment. As a consequence, this lowers the reliability and utility of variety descriptions and makes them biased for identification purposes in the global marketplace. Furthermore, the variety description for a ryegrass or sugar beet variety will not give precise discrimination between varieties whether they include only UPOV traits or not.

Accelerated crop improvement

Plant breeding aims to combine traits of interest in one inbred line (e.g. for inbred cereal crop varieties) or to increase the frequency of favourable alleles within an outbreeding population (e.g. alfalfa). However, this achievement is a lengthy process. For example, it can take up to 15 years for the breeding of a cereal crop variety, from crossing desired parents and inbreeding through segregating populations (3–7 years) to testing for yield, disease resistance, and quality traits across multi-environmental trials (4–5 years) and finally seed increase and release of the improved variety (1–3 years). Similar timelines exist for the development of hybrid cultivars of an outbreeding species like maize, where selection of desired parents and the inbreeding process takes up to 10 years (Shimelis and Laing 2012).

A recent innovation in accelerating improvement of plant varieties, 'speed breeding', permits growing up to 6 generations per year compared to just 2–3 generations in a regular glasshouse or 1–2 generations in the field. For most day-neutral or long-day species, such as spring wheat, durum wheat, barley, oat, chickpea, pea, and canola, the approach involves growing plants under extended photoperiod (22 h light) and controlled temperature conditions (Watson et al. 2018). The technique can be performed using growth chambers or scaled-up to large glasshouse facilities fitted with LED supplemental lighting (Ghosh et al. 2018). This tool has already enabled peanut breeders to halve the time required to develop new varieties from 10–15 to 6–7 years (O'Connor et al. 2013). A similar approach using 20 h of light permits legume breeders to accelerate the single seed descent method and enables growing 6–8 generations of lentil, chickpea, field pea and lupin per year (Croser et al. 2014), compared to 1–3 generations per year using conventional methods. Speed breeding protocols have also been developed for short-day crops, such as rice, soybean and amaranth (Jähne et al. 2020). It should be noted that while breeders can circumvent the need for the development of recombinant inbred lines over successive generations of inbreeding via the use of doubled haploid technology and achieve inbred lines in a year, this approach is relatively expensive and restricted to specific germplasm or specific crops (Kalinowska et al. 2018).

⁴ Sugar beet, *Beta vulgaris* L., Inclusion in Dutch register of varieties, <https://www.naktuinbouw.com/agriculture/variety-description/sugar-beet-beta-vulgaris-l>.

Plant breeders have recently deployed molecular tools to assist the selection of desirable traits in promising lines. Molecular markers diagnostic for, or linked to, genes of interest have been used in marker-assisted selection of desired genotypes and pyramiding beneficial alleles at two or more genes into one cultivar. The advent of next-generation sequencing (NGS) methods has underpinned efficient genotyping systems, such as genotyping-by-sequencing (GBS) or single nucleotide polymorphism (SNP) marker platforms. In addition to their application for genetic trait dissection and identification of marker-trait associations in plants, the costly (in terms of time and money) phenotyping phase can be partly reduced via the use of GS, whereby breeding values of plants or lines are predicted using a large number of molecular markers and models developed using a phenotyped ‘training population’ (Crossa et al. 2017). Whilst GS can shorten the breeding cycle via the advancement of a set number of generations without the need for phenotyping, the development of improved varieties could take place at an even faster pace through ‘SpeedGS’, a combination of GS with speed breeding (Voss-Fels et al. 2019; Hickey et al. 2019).

Why accelerating crop improvement is crucial

Farmers will need to produce an estimated 70% more food by 2050 in order to feed 10 billion people (United Nations 2017). To achieve this goal, plant breeders are striving to develop more productive crop varieties, particularly because arable land is expected to decrease from 0.25 hectare per capita in 2010 to less than 0.2 per capita in 2050 (Bruinsma 2009). Moreover, future crop varieties must be more resilient in the face of climate change and rapidly evolving pests and diseases.

A recent 15-year study on the impact of plant breeding shows that three quarters of overall crop productivity growth in the EU is due to improved cultivars of just nine major crops. This contribution is also supported by a 1.24% increase in yield per year (Noleppa 2016). In detail, the significant contribution of genetic gain is shown by eliciting data from official VCU trials. Rijk et al. (2013) studied genetic progress of newly released winter wheat, spring barley, potato (ware and starch types) and sugar beet varieties on the official Dutch variety list between 1980 and 2010 and

found a linear increase in yield of these main crops. Analyzing official variety trials of spring (barley, oat, wheat) and winter (rye, wheat) crops conducted in Finland during 1970 to 2005 demonstrated a good contribution of improvement due to plant breeding activities (Peltonen-Sainio et al. 2009). Similarly, in the United Kingdom, Mackay et al. (2011) showed that since 1982, 88% of the improvement of yield in cereal (winter wheat, spring and winter barley) and oilseed rape varieties was due to genetic effects. In Norway, 890 VCU spring barley trials were assessed in three sub-periods from 1946 to 2008 where 29, 43 and 78% of yield improvements were due to genetic effects (Lillemo et al. 2010). Laidig et al. (2014) found that progress in yields of 12 major crops in German VCU trials over a 30-year period (1983–2012) was due to genetic gains. Moreover, Bornhofen et al. (2018) found 1% per year genetic gain of Brazilian wheat varieties by analyzing yield data of 836 VCU trials conducted between 1998 and 2014.

It is conceivable that speed breeding may open up exceptional opportunities to hasten the trends of genetic gain in crop varieties via combination with diverse plant breeding tools (Hickey et al. 2019).

Could modifications of the DUS testing process help hasten variety release?

Considering the positive impact of speed breeding protocols on reducing the timeframe of plant breeding, variety registration is lengthy and time-consuming enough to delay the release of improved varieties to farmers. This is especially the case for crop varieties, as they should pass additional performance tests (VCU) in addition to DUS, in order to be eligible for registration in national lists of varieties. Given these two testing procedures, the whole variety registration process can take from 2 to 4 years, depending on different scenarios (Fig. 1). Firstly, when the DUS and VCU tests are carried out in parallel (but separate) trials, the whole process takes 2 years. But, there is always a risk of rejection at the end of second year owing to lower performance of candidate varieties compared to the standard (check) varieties in VCU trial. For example, only up to one-fifth of varieties from 30 crop species annually entered to official VCU trials are permitted for release in Germany (Laidig et al. 2014). The figure for rye is roughly three

approved varieties out of 20–30 candidates entered into VCU trials annually (Laidig et al. 2017). Therefore, it is reasonable that breeders delay DUS tests for 1 year when possible until they establish whether the performance of their candidate varieties are sufficiently promising at the end of VCU tests in first year. This approach extends the registration process to 3 years. Also, there would be some instances where the results of the performance testing step (as an integral part of breeding program) is equivalently accepted as VCU by the variety release committee. This is an approach that may be adopted in countries with predominantly public sector breeding, and is typically followed by 1 year of on-farm trials. This scenario represents another reason for delaying DUS tests until public breeders get feedback on the performance and adaptation of crop varieties in different agro-ecological conditions. Notably, the whole process may be extended to 4 years, where DUS testing is done after completion of VCU testing (Turner and Bishaw 2016). In some countries, the procedure would be lengthier for certain crops. In the United Kingdom, VCU trials for herbage crops take 4 years (instead of, for example, the 2-year VCU programme for annual crops)⁵ and in Germany 3 years is needed for evaluating performance of cereal, winter oilseed rape, and fodder grass varieties (Laidig et al. 2014). On the other hand, in countries with no mandate for variety registration before release (e.g. Australia) or vegetables and certain crop varieties (e.g. in Canada), DUS testing is the cornerstone of the PVP system by establishing the unique identity of varieties.

It is reasonable to speculate that changes to the current registration process could result in improved varieties being delivered more quickly to farmers, and a variety of recommendations could be given. For VCU testing of crop varieties, it has been suggested that performance data of released varieties in similar agro-ecological conditions could be shared between examination offices in different countries, provided similar standards and protocols were used (Turner and Bishaw 2016; Gilliland et al. 2020). As mentioned previously, another option could be that variety release committees accept the performance testing step of

public plant breeding as equivalent to VCU trials, provided that they follow a compatible protocol. More importantly, we explain below the possibility for shortening duration of conventional DUS testing, as it is an essential component of variety registration. In addition, we compare the attributes of each of these methods for addressing limitations in the number of traits, cost, changeability due to environmental effects, discriminating power and suitability for ‘Uniformity’ and ‘Stability’ assessments, as well as labour-intensity (Table 1).

Possible approaches for accelerating DUS testing

Speed DUS testing

DUS testing is normally completed over two independent growing cycles in the field or glasshouse (e.g. for annual crops). The resulting variety descriptions provide plant varieties with an ‘identity’ in respect to phenotypic characteristics. However, changeability of quantitative traits in different environments can reduce their utility and reliability, e.g. describing flowering time of a variety as ‘early’ is relative to the example varieties grown and can be dependent on growing environment. This brings disadvantage to examination offices that take over DUS reports from other testing authorities instead of conducting DUS tests themselves.

DUS testing of field crop varieties could be conducted under controlled conditions to lessen the effect of environment. It has been demonstrated that some morphological traits in wheat and barley can be faithfully phenotyped under speed breeding conditions, whereby phenotypic variation for awns (presence/absence), plant height (short/medium/long), flowering time (early/late) in wheat, and leaf glaucosity (very weak/weak/medium/strong) in barley is parallel to UPOV’s characteristics nos. 17, 13, 7 (UPOV 2017a), and 8 (UPOV 2018a) in DUS test guidelines (Watson et al. 2018). These examples show that at least some DUS traits are amenable to characterization under ‘rapid generation advancement’ conditions. This would allow tests to be undertaken simultaneously in latter steps of speed breeding, or independently over a much-reduced time frame than the current 2 years required. In the first instance, more research is required to calibrate

⁵ How to apply for national listing of agricultural and vegetable plant varieties in the UK, Department for Environment, Food & Rural Affairs, the UK, <https://www.gov.uk/guidance/national-lists-of-agricultural-and-vegetable-crops>.

Table 1 Comparison of four approaches with conventional testing

	Conventional testing	Speed DUS testing	Diagnostic markers	Combined phenotypic and genetic distances	Varietal description by DNA sequencing
Test type	Morphological	Morphological	Molecular	Morphological + Molecular	Molecular
Limitation in number of traits/markers	High	High	Medium	Low	Low
Relative financial cost	High	Low to medium	Low	Medium	Low
Changeability due to environmental effects	Low (qualitative traits) to high (quantitative traits)	Low	Low	Low (qualitative traits/DNA markers) to high (quantitative traits)	Low
Discriminating power (D assessment)	Low to medium	Low to medium	Low to medium	High	High
Suitability for ‘U’ and ‘S’ assessments	High	Medium	Medium	NA	High
Labour-intensity	High	Medium	Low	Medium	Low
Duration of test	2 years	Less than 1 year	A few weeks/months	1 to 2 years	A few weeks/months
Practical examples	Numerous	–	Tomato (Arens et al. 2010); barley (Cockram et al. 2009, 2010, 2012)	Soybean (UPOV 2018b); maize (UPOV 2017b); potato (UPOV 2011)	Maize (Tian et al. 2015; Hall et al. 2016); alfalfa (Annicchiarico et al. 2016); wheat (Gao et al. 2016; Singh et al. 2019)

NA not applicable, as the method deals only with Distinctness

expression of DUS traits in example varieties of common knowledge. Then, DUS data of candidate varieties could be compared with similar varieties or checked against a repository of variety descriptions (maintained by UPOV or examination offices) for final decision on ‘Distinctness’. It is also feasible to test for ‘Uniformity’ and ‘Stability’ of new plant varieties by growing required number of plants under two successive rapid generation advancement conditions.

Diagnostic markers

Recommended under UPOV Model 1, “*Characteristic-specific molecular markers*”, genetic markers diagnostic for target DUS traits could be used to predict phenotypic states. Currently, a handful of diagnostic markers are available to DUS examiners. Arens et al. (2010) introduced a number of linked markers to disease resistance genes and suggested that

they could be robustly used as an alternative or complement molecular assay to current labour-intensive tests used in ‘Distinctness’ testing of tomato varieties. In barley, diagnostic markers have been developed for evaluation of winter/spring seasonal type (Cockram et al. 2009) and ear row number (Cockram et al. 2012), and linked SNPs predictive of phenotype are available for a variety of additional barley DUS traits (Cockram et al. 2010; 2012). While such markers could be efficiently utilized in ‘Distinctness’ testing, their successful application in evaluating ‘Uniformity’ has received little attention to date. However, a recent proof of concept study in barley has shown that where diagnostic Kompetitive Allele-Specific PCR (KASP) genetic markers are available, these should be sensitive enough to robustly detect off-types in a single DNA pool composed on one off-type in a pool of ten individuals (Saccomanno et al. 2020). This illustrates the potential of using molecular

markers to test all three components of the DUS criteria. However, while such methods are workable in self-pollinating and vegetatively-propagated species such as cereals and potato, it is unlikely to be applicable for population-based varieties of outbreeding species (e.g. ryegrass) owing to heterogeneity between individual plants (Wang et al. 2016). Prospects for development of diagnostic markers significantly depends on understanding of molecular mechanism underlying DUS traits. Accordingly, genome-wide association studies across a diverse panel of plant varieties is a workable approach to elucidate the genetic architecture of complex DUS traits, e.g. plant height, and decipher not only their causative loci/genes but ultimately the causative sequence variants. Nevertheless, identification of all the genetic loci and variants that control any given DUS trait, especially those assessed on a more quantitative scale, is a considerable challenge.

Combined phenotypic and genetic distances

In some species, selection of comparable varieties sampled from large reference collections is a costly and labour-intensive task. In China, side-by-side comparison of more than 6000 registered maize varieties with candidate lines in the field is a major challenge for DUS examination (Tian et al. 2015). Similar scale is for maize in France, with around 4000 inbred lines and 4000 hybrids tested in side-by-side comparison (UPOV 2014). Based on UPOV's Model 2, "*Combining phenotypic and molecular distances in the management of variety collections*", only closely similar comparable varieties to candidates are entered in the field for comparison (UPOV 2013). Implementation of this approach has led to considerable reduction in costs and workload for DUS trials. Having halved the number of field comparisons in soybean (UPOV 2018b), there would be as much as one million variety pairs of maize if the 'super distinct' (beyond a morphological threshold) and 'distinct plus' (beyond morphological and molecular thresholds) check lines are not eliminated by this approach (UPOV 2017b). Also, there is no necessity to test 'super distinct' potato varieties in a second year of DUS testing, as their morphological distances from already registered varieties is much beyond the pre-defined threshold (UPOV 2011).

Varietal description by DNA sequencing

Many plant breeders are in favour of modernizing PVP in such a way that morphological descriptions are replaced by DNA sequencing data. This would deliberately reduce DUS testing time of new plant varieties from a lengthy 2 years to just a few weeks/months. However, two major concerns include the reduction of minimum genetic distance between varieties and the irrelevance of the majority of DNA variation to existing phenotypic DUS traits. These concerns would be real if an insufficient number of anonymous markers were used to demonstrate distinctness between varieties. The consideration of DNA markers for use in DUS testing dates back to the 1990s, when randomly amplified polymorphic DNA (RAPDs) and amplified fragment length polymorphisms (AFLPs) were deployed in distinguishing oilseed rape and wheat varieties, respectively (Lee et al. 1996; Law et al. 1998). Since then, mostly AFLPs and simple sequence repeats (SSRs) have been used as complement or substitute for DUS traits in varietal characterization of agricultural crops, vegetables, fruits and forest trees (for a review see Jamali et al. 2019). The vast majority of markers used were unrelated to DUS traits, though in some instances two AFLP primer combinations were informative enough to delineate 83 oilseed rape cultivars based on seasonal growth habit (winter versus spring), breeding company and country of origin (Lombard et al. 2000). Thanks to the availability of diverse and low-cost NGS platforms, a paradigm shift from conventional markers to SNPs could occur for varietal identification. SNPs are highly abundant in the genome of plants and can be easily assayed and automated for high-throughput sampling. Recent examples of SNP fingerprinting maize (Tian et al. 2015; Hall et al. 2016) and alfalfa (Annicchiarico et al. 2016) accessions demonstrate the potential of SNPs for the molecular barcoding of new plant varieties. In wheat, a set of 43 SNPs has been shown to provide unique barcodes capable of discriminating 429 cultivars sourced from across China (Gao et al. 2016). The complementary usefulness of a similar number of SNP markers were recently shown in the identification of 368 Indian wheat varieties using a web-based comparison tool (Singh et al. 2019). Recent application of SNP markers in seed certification of barley control plots (Owen et al. 2019) could also be combined with the aforementioned approaches

to speed up delivery of improved varieties to farmers. While this novel approach alleviates the cost and lengthiness of the certification process, it also highlights the usefulness of DNA markers in all steps of plant variety development chain.

Conclusion and future prospects

The examples discussed in this article were drawn primarily from crop varieties released in countries which require mandatory tests to be satisfied (DUS and VCU) before release. Overall, DUS standards are the cornerstone of variety registration as they provide unique identity for each marketed variety.

A number of DUS testing systems have been implemented in different countries. Varieties can be officially examined with centralized testing or commissioned to testing stations. Within this system, which is adopted in most EU countries, basic variety information is extracted from submitted applications and technical questionnaires. Also, there would be a cost-efficient option that candidates be connected to a whole range of varieties within inspection trials (pre- and post-control plots) of seed certification process. This approach would avoid the necessity for duplicate sowing of the reference collection for comparison. In other systems, breeders can contribute to DUS testing (e.g. France) where, for instance maize varieties are tested at breeder premises in first year followed by official testing as a second cycle. The tests could be monitored by official examiners (e.g. Australia, Canada, Japan) or conducted by breeders themselves. The so-called ‘breeder testing’ system is employed in the USA: breeders seeking PBR (under the PVP Act of 1970) provide DUS Distinction information for their candidate variety to one or two of the most similar varieties, whereupon examiners verify the information through searching databases of variety descriptions. The uniformity and stability of varieties are not assessed by examiners, and the onus of DUS is placed on the breeder (Van Wijk and Louwaars 2014). Additionally, in Canada it is the responsibility of breeders to provide variety descriptions of candidate varieties for registration before approval for marketing.

We propose the ‘speed DUS testing’ approach, which is in favour of a breeder testing system through maintaining current phenotype-based DUS standards.

Within this approach, DUS testing of crop varieties could theoretically be conducted under rapid generation advancement conditions across a much-reduced timescale of months rather than years. In addition, costly and labour-intensive comparisons in the field could be replaced by providing a description of promising varieties against similar varieties or a repository of calibrated variety descriptions. Another advantage is increased reliability of variety descriptions - crucial for identification purposes in the global marketplace. Speed DUS testing would offer many benefits for public testing institutions, however, potential drawbacks to the adoption of this approach could be access to suitable facilities, and the possible need to reduce the number of DUS traits tested, as some traits may not be robust under speed DUS testing conditions. Even if time savings can not be achieved by implementing speed DUS testing, the procedures would still provide considerable benefits to enhance the precision of DUS testing, as required by variety registration and protection.

In contrast, alternative methods take advantage of DNA markers and/or sequencing information as a complement or substitute for DUS traits. Such approaches would require examination offices that currently employ phenotype-based DUS standards to accommodate genotyping data in variety registration. Also, this provides a fast and robust data where identity of varieties should be determined in cases of mix-up or delivery errors in seeds, as well as PBR infringements. Nevertheless, the critics of existing PVP systems are in favour of conceptualizing plant varieties as genetic datasets in the era of technological change rather than phenotypic descriptions (Janis and Smith 2007).

We envisage that altering the DUS testing procedure could reduce the duration of variety testing. For instance, the registration process could be reduced to 1 year in ‘combined phenotypic and genetic distances’ or even few weeks via ‘diagnostic markers’ or ‘describing by DNA sequencing’ approaches, whilst addressing shortcomings of conventional testing (Table 1). However, with the increasing number of speed-bred varieties, there is an argument in favour of narrowing the gap between conventional registration procedures and fast-tracking crop improvement. Until this happens, lengthy DUS accreditation processes may in some cases hold back the full benefit of new

breeding approaches that aim to accelerate the delivery of improved crop varieties.

In the era of precision breeding, a genome edited-crop may not necessarily satisfy DUS criteria if bred for a utility or VCU trait. In other words, VCU traits are not capable of precise description of varieties as they are significantly influenced by environmental factors. For instance, a wheat plant with tolerance to pre-harvest sprouting, which is a crucial trait at harvest time in wet areas, could be developed through knocking out the endogenous *TaQsd1* gene. The edited wheat differs from its founder variety in just three nucleotides, consisting one point mutation at each of the homoeologous *TaQsd1* genes on the A, B, and D sub-genomes (Abe et al. 2019). As the resulting resistance to pre-harvest sprouting conferred is a VCU component rather than a DUS trait, existing protocols would not identify the gene-edited line as DUS Distinct. Here, marker or sequencing information can play a role in linking DUS and VCU testing, as envisaged earlier by Cooke and Reeves (2003). Another bottleneck that may hamper release of varieties is pre-market risk evaluations. In particular, Canada is a unique country that scrutinizes biosafety of 'plants with novel traits' within its territory, irrespective of the breeding technique (conventional or modern) employed.⁶ Such trials are a prerequisite for variety registration (Fig. 1), but it has been suggested that their data can be shared for compatible VCU assessments to avoid repetition (Slot et al. 2018). On July 2018, the EU's European Court of Justice ruled that crops developed from gene editing technology should be regulated as genetically-modified crops (Callaway 2018). Therefore, gene-edited crops must pass analogous safety trials originally designed for genetically-modified crops prior to marketing. This further delays marketing of improved varieties by 2–3 years, which is in addition to the combined DUS and VCU testing process that takes 2–4 years.

The advent of speed breeding protocols has dramatically reduced generation time in many plant species. This allows for growing multiple generations per year for broad range of day-neutral, long-day and short-day species (Watson et al. 2018; Jähne et al.

2020). Combining these protocols with technologies like MAS, GS and NGS results in fast-tracked development of new varieties. For example, integration of speed breeding within a rice MAS backcrossing program resulted in the development of a salt-tolerant variety in only 17 months. The new variety was 93.5% similar in agronomic traits to the susceptible recurrent variety, while harbouring the donor gene conferring tolerance to salinity and its flanking genomic region (Rana et al. 2019). However, the challenge remains that the improved variety fails to satisfy DUS criteria, even though it performs better than check varieties for salinity tolerance within VCU trials. Such examples highlight the need for reviewing current regulations and policies of countries in variety registration, particularly those pertaining to DUS testing. In that respect, modern approaches such as speed breeding and DNA-based variety characterization would play an expeditious role in the future of variety registration provided that their importance and priority are appreciated by policy makers, particularly in the face of climate change and the associated challenges on global food security.

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Compliance with ethical standards

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

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