UNIVERSITY OF HOHENHEIM

Advancing soybean adaptation to Central European growth conditions with novel breeding tools

Dissertation to obtain the doctoral degree of Agricultural Sciences (Dr. sc. agr)

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submitted by

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List of Abbreviations

CE	Central Europe
DH	double haploid
DNA	deoxyribonucleic acid
EU	European Union
FT	flowering locus T
GM	genetically modified
GS	genomic selection
LD	long day
LED	light emission diode
MAS	marker-assisted selection
MG	maturity group
N	nitrogen
QTL	quantitative trait locus
SD	short day
SSD	single seed descent
TKW	thousand kernel weight

General Introduction

The soybean (*Glycine max* (L.) MERR) is one of the world's most important annual crops. It was domesticated from its wild progenitor *Glycine soja* (SIEB. et ZUCC.) between 2500 and 4000 B.C. in Central China (Wang et al. 2010). Among all crops it ranks 6th regarding global production (348.7 mio. tonnes), 4th regarding worldwide acreage (124.9 mio. hectares) and 1st by far in both categories if only legumes are concerned (FAOSTAT, 2018). Soybeans are an important component in the human food and animal fodder industry due to their high nutritional value originating from a high protein content, high micronutrient concentrations and high levels of unsaturated fatty acids (Smith et al. 1972). Soybeans possess the ability to form rhizobia based on mutualism with *Bradyrhizobium japonicum* (Keller et al., 1999). Therefore, like other legumes, it is able to fixate atmospheric nitrogen (N). Studies have shown that, although additional N-fertilisation on soybean fields in low-yielding environments has positive yield effects, the majority of the plant's N-demand is covered by biological N fixation (Salvagiotti et al. 2008). Consequently soybean only requires a reduced application of N fertiliser. Hence, it is a valuable component for crop rotation cycles. These attributes aid to increase soil fertility and decrease infestation risks caused by soil-borne pests, diseases and narrow crop rotations, which demonstrates the agronomical value of soybeans.

Figure 1 Pictures of soybean plants, seedlings, an axial flower and mature pods. Photographed by V. Hahn, A. Kurasch, M. Haupt.

Soybeans in Europe

Worldwide, the vast majority of soybeans are harvested on the American continents (86.7 %, FAOSTAT 2018). Europe's global production share of 3.5% is comparatively low, of which only 23.5% are soybeans grown on soils of the European Union (FAOSTAT 2018). Although the global market shares of EU soybeans are marginal, in 2018 overall soybean production rose by 14.6 % compared to the 5 year average reference period, while simultaneously the overall production of oilseed crops declined by 11% (EU Commission 2020, ec.europa.eu). During this time period soybean acreage in Germany increased almost 5-fold from 5000 ha in 2013 to 24000 ha in 2018. In 2018 EU member states produced 2.9 mio. t of soybeans. Top producer was Italy (1.14 mio. t, 39 %) followed by Romania (16 %), France (14 %), Croatia (8 %), Austria and Hungary (both 6 %, Eurostat 2020). The increase of soybean production might have profited from various EU programs which aimed to promote the agricultural cultivation of legumes (i.e. EUROLEGUME, LEGATO, Legume Futures) and from national and international formations of soybean networks and organisations (i.e. Soja-Förderring, Donau Soja, Europe Soya). Fifteen EU member states have signed a European Soy Declaration in order to increase cultivation of legumes, embedded in national crop strategies (Czaplicki Cabezas et al. 2019). However, the recent European soybean boost cannot conceal the gargantuan "hunger" for soybean products and the consequently tremendous deficit between EU's own production and its needs: In 2018/19 the already stated EU-wide production of 2.9 mio. t of soybeans faced imports in the amount of 32.9 mio. t. (15.1 mio. t soybean seeds and 17.8 mio. t soybean meal, EU Commission 2020, ec.europa.eu). In order to increase soybean production to a more self-sufficient and sustainable level, soybean yield must be increased and growing areas must be expanded. This however is not a trivial endeavour since there are considerable challenges involved including foremost day length adaptation and cold tolerance of an originally subtropical plant adapted to short-day (SD) conditions and warm temperatures. The following chapter will elucidate the most important soybean breeding objectives for Central Europe (CE) more in detail.

Breeding objectives for soybeans in Central Europe

Soybean is a typical self-pollinating crop with on average 1.8 % natural outcrossing rate under field conditions (Ray et al. 2003). Reports about the value of heterosis in soybeans and consequently its successful exploitation through the use of F1-hybrids are conflicting (Brim and Cockerham 1961, Weber et al. 1970, Lewers et al. 1996, Cerna et al. 1997, Zhang et al. 2017). To date there seems to be little knowledge and research about double-haploid (DH) production of soybeans. Rodrigues et al. (2005) stated general difficulties in various studies to obtain strong and healthy plants from calli generated from anther cultures.

Furthermore, genome-edited soybeans only recently started to become a subject of research or an element of corporate interest (Li et al. 2015, al Amin et al. 2019, Bao et al. 2019, Metje-Sprink et al. 2020). However, due to consumer demands and European legislation, genetically modified (GM-) crops are less an option for the EU market. All this is why pedigree breeding for lines is still the most common approach to release novel and improved soybean cultivars. The pedigrees of the majority of soybean lines that are adapted to CE climate conditions trace back to plant material from North America (Hahn and Würschum 2014) and belong to early maturity groups. This maturity group system from very late (MG XI) to early (MG 0) to very early (MG 000) was established in the USA and Canada and is based on the cultivar's photoperiodic sensitivity and its ability to mature within the vegetation period of a certain geographic latitude. Over the last decades and despite the narrow genetic basis, tremendous improvements were achieved to enable the growth of soybeans under cooler, long-day (LD) conditions of CE. Efforts to continue this adaptation process are still under way and focus on the following traits:

Photoperiod and Maturity

Soybean's wild progenitor *Glycine soja* (SIEB. et ZUCC.) is a subtropical, photoperiod sensitive short-day (SD) plant and flowers only if the ambient night period is long enough (Hamner 1940). Cultivation in higher latitudes with LD conditions and a shorter vegetation period requires a certain level of photoperiodic insensitivity because otherwise flowering and maturity cannot be completed within the available growing season. The discovery of photoperiod insensitive mutants was an important step to widen the cultivation of soybeans to more northern areas. Holmberg (1950) described the early maturing Swedish cultivar Fiskeby which was selected in and for the cold Swedish climate in high latitudes. Also Yoshida (1952) reported early maturing soybeans in Japan which were not sensitive to photoperiod. This variation in photoperiodism was confirmed by several studies (Criswell and Hume 1972, Mayor et al. 1975, Hadley et al. 1984). The molecular basis for the observed variation lies within mutated maturity genes. To date, 12 maturity genes from *E1* to *E11* (Bernard 1971, Buzzell 1971, Buzzell and Voldeng 1980, McBlain and Bernard 1987, Bonato and Vello 1999, Cober and Voldeng 2001, Cober et al. 2010, Kong et al. 2014, Samanfar et al. 2017, Wang et al. 2019) as well as the *J* locus for long juvenileness (Ray et al. 1995) are discussed, though only a few of them contribute confirmed major effects to maturity in higher latitudes (Tsubokura et al. 2014, Cao et al. 2017, Miranda et al. 2020). Central to the regulatory pathway of flowering initiation in soybeans is *E1*, a suppressor protein of the flowering locus T (*FT*) (Xia et al. 2012a, Xia et al. 2012b, Tsubokura et al. 2014). Mutations in the *E1* locus enable early flowering and seed maturity independently of the day length. The *e1* genotype (*e1-nl, e1-as, e1-fs*) is found abundantly in soybean plant

material grown in high-latitude environments (Tsubokura et al. 2014, Jiang et al. 2014) and almost exclusively in European material (Kurasch et al. 2017). The other major maturity genes *E2*, *E3* and *E4* play important regulatory roles for *E1* or *FT* as well and therefore also in early flowering and maturity of soybeans for northern growing areas (Watanabe et al. 2009, Watanabe et al. 2011, Xia et al. 2012a, Tsubokura et al. 2014, Cao et al. 2017, Kurasch et al. 2017). By combining these early-maturity alleles, very early and extremely early maturing soybeans (MG000 and MG0000) were obtained from European breeding programs. These cultivars are able to fully complete their life cycle in 21 weeks in environments between 51° and 54° latitude north while other material of later maturity groups (00 - II) failed to do so (Kurasch et al. 2017). When grown in more southern areas at 45° latitude north, the same MG000 material could reach maturity even faster within 16 weeks and additionally also the maturity groups MG00 to MGII ripened on average within 18 weeks. Future improvements on matters of early maturity are to be expected due to growing interest of private breeding companies and consequently increased budgets for soybean breeding programs targeting European growing sides.

Cold tolerance

Soybeans, by origin, favour warm, subtropical climates. Exposure to cold weather can lead to decelerated growth or shedding of flowers and pods and therefore reduced productivity (Takahashi and Asanuma 1996, Funatsuki and Ohnishi 2009, Ohnishi et al. 2010, Toda et al. 2011). But similar to former photoperiodic restraints, soybeans have agriculturally crossed a temperature boarder too, now grown in Canada, Scandinavia and Hokkaido, far beyond subtropical areas. A few decades ago variation towards tolerance of cold temperatures was observed in two developmental stages: during seed germination and emergence (Littlejohns and Tanner, 1976) and during anthesis and early pod formation (Hume and Jackson, 1981; Schor et al. 1993). Keeping in mind the aim to expand the soybean growing area to higher latitudes, one needs to be aware of an increased risk of late frosts after sowing or cold night temperatures below 15°C during early plant development. Data from the German Climate Data Center (Deutscher Klimadienst, 2000 – 2019, dwd.de) confirm a delayed start of the vegetation period in the north-east compared to the south-west of Germany. Consequently a spring-temperature gradient from south-west to north-east can be expected beyond the German borders, as well. This is why, in order to push cultivation of soybeans northwards and exploit areas with shorter vegetation periods, soybean material with a tolerance towards chilling stress is required, especially in the early developmental stages when cold temperatures occur and soybeans are susceptible to cold temperatures (Balko et al. 2014). The molecular basis for cold tolerance in soybeans is not yet completely understood but studies exist that were able to show a correlation with some loci, e.g. with

the pubescence colour *T* locus, major maturity alleles or plant height *Dt1* locus (Takahashi et al. 2005, Funatsuki and Ohnishi 2009). Hume and Jackson (1981) as well as Yamagushi et al. (2018) stated in their experiments that the most cold-tolerant genotypes in their panels derived from pedigrees that incorporated either Fiskeby V or Fiskeby 840-7-3. Despite these studies, efforts are worthwhile to further understand the molecular mechanisms and involved molecular pathways of cold tolerance in soybean material that is already grown in CE. A deeper understanding of the genetic basis of these processes will be valuable to choose parental genotypes for breeding programs that aim for soybean cultivation in northern parts of CE.

Disease resistance

So far, the soybean – due to its still "new" agricultural status and the small acreage – is a robust and healthy crop in CE (Deutscher Sojaförderring e.V., https://www.sojafoerderring.de/). While photoperiod insensitivity and cold tolerance are the most important traits in adapting soybeans to CE conditions, agronomically important soybean diseases are considered nonetheless. Undoubtedly these will further spread (sklerotinia, rhizoctonia, diaporte/phomopsis) or can even arrive in EU (soybean cyst nematode) once soybeans are integrated into the cropping systems on a larger scale. To date, good farming practice, e.g. at least 3 year crop rotations (reduces infestation with sklerotinia) or proper tillage and a well-chosen sowing date (reduces risk of rhizoctonia) seem sufficient to keep soybeans healthy. There is, however, knowledge of some molecular mechanisms and quantitative trait loci (QTL) marker that increase resistance to soybean cyst nematodes (Yang et al. 2017, Cook et al. 2014, Liu et al. 2012), the diaporte/phomopsis complex (Mena et al. 2020, Pioli et al. 2003), or resistance to sklerotinia (Kim and Diers 2000), that could be employed for marker-assisted selection (MAS) in breeding programs. Breeders can incorporate these traits into their programs to increase basic resistance of the material instead of breeding for qualitative resistances once a disease has taken root that is then again quickly overcome in a typical boom-and-bust cycle. To some extent, sealed climate chambers can be used to test the material for resistances indoors. The advantage is twofold: conditions can be ideally set to ensure an optimal infestation (often not met in the field) and the pathogen is kept off the field.

Yield

A crop as adapted as can be to photoperiod or temperature conditions will not establish on the market if farmers cannot get a proper yield off it. Surely a crop will not realise its full yield potential or maybe nothing at all if those criteria are not met or if a field is heavily devastated by pathogens. But in the end, yield is the trait it all boils down to – often also in plant breeding.

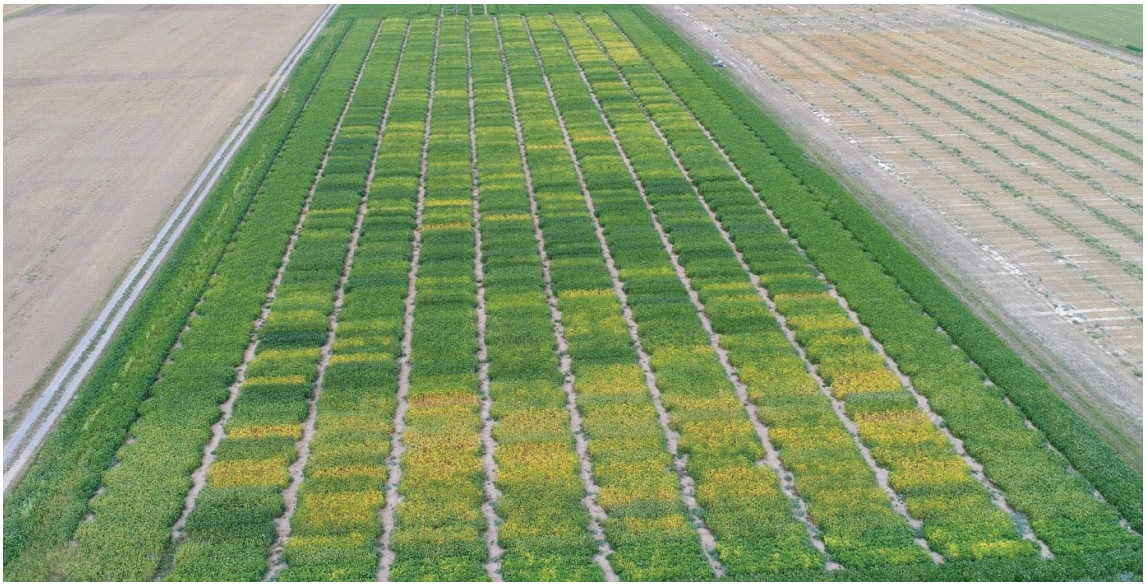

Figure 2 Drone picture of soybean yield test plots on Heidfeldhof (University of Hohenheim) in September 2018 with observed substantial variation in maturity. Photographed by M. Haupt 2018.

Like in other crops, the genetic architecture of soybean yield and its components are highly quantitative and therefore hardly understood in detail (Yuan 2002, Xavier et al 2016, Diers et al. 2018). Consequently, no loci with large effects can be expected that could be used in MAS. The concept of genomic selection (GS) (Hayes and Goddard. 2001) has been evaluated for plant breeding as a way to capture the many minor effects based on the genomic information and draw selective conclusions from it (Heffner et al. 2009; Tester et al. 2010; Spindel et al. 2015; Marulanda et al. 2016). GS was applied to soybean breeding as well (Jarquin et al. 2014, Zhang et al. 2016, Duhnen et al. 2017) with robust prediction accuracies between 0.62 and 0.64 for grain yield or grain yield components, respectively. Reports about a correlation between high yield and high maturity group are contrasting: Egli et al. (1993) argued that early maturity soybeans have an equally high yield potential as material from higher maturity groups. Kabelka et al. (2004) reported both, higher-yield-QTL that are strongly associated with longer maturity but also yield-QTL that are not specific to maturity group. Some studies on the other hand observed a high correlation of increased yield and maturity time possibly due to linked or pleiotropic effects (Guzman et al. 2007, Liu et al. 2011, Diers et al. 2018), which would impose challenges for breeding high-yielding cultivars for northern growing areas under LD conditions with short vegetation periods. But even within the early maturity segment (MG 0 to 000), yield advanced by on average 13 kg per hectare per year for soybean cultivars released between 1976 and 1992. This increase displays an average annual selection gain of 0.7 % for yield (Voldeng et al. 1997, Morrison et al. 1999). Given that this value was achieved through conventional soybean breeding

which takes more than a decade from the initial cross to the market release of the new cultivar, the annual selection gain could increase by shortening the breeding cycle. Consequently, this would elevate the release frequency of novel and improved cultivars. As already stated earlier, many breeding instruments (DH, genome editing) are no option for European soybean breeding due to technical or political hurdles. GS is one possible instrument to accelerate plant breeding through early generation selection of quantitative traits. Moreover, the concept of speed breeding could be used to breed for improved cultivars adapted to CE conditions more quickly. The following chapter elucidates this concept more thoroughly.

Speed breeding under controlled conditions

Speed breeding (Watson et al. 2018) was introduced for LD crops. Plants are kept in a greenhouse and are exposed to artificially elongated day lengths of 22 hours and to a short night break of 2 hours, thereby spurring on vegetative and generative development of the plant. In combination with a premature seed harvest and subsequent drying of the immature seeds it reduces the generation time of a LD crop significantly. Watson et al. (2018) showed that they were able to grow 6 generations of spring wheat, barley and chickpea and 4 generations of canola within one year this way. Given the equation for genetic gain from selection

$$\Delta G = \frac{i * h * \sigma_A}{L} \qquad (1)$$

i = *selection intensity*

h = *square root of the heritability*

σ_A= *square root of the additive genetic variance*

L = *length of breeding cycle*

Li et al. (2018) and Hickey et al. (2019) proposed to increase genetic gain by a combination of methods: Increasing the genetic variance component σ_A i.e. by genetic screening of gene bank material to exploit maximum genetic variation, and decreasing the length of the breeding cycle *L* i.e. by GS and accelerated backcrossing or reduced generation time through speed breeding. Setting optimal temperatures or the adjustment of watering and humidity are crucial parameters when operating speed breeding (Ghosh et al. 2018), which is why indoor artificial conditions are proposed. As opposed to the fixed 22 hour day length for speed breeding LD crops, the control of day length conditions plays a crucial role in adapting speed breeding to photoperiod sensitive SD crops because under extended day length conditions there will be no or only delayed flowering. To date, knowledge is scarce

about critical day length adjustments for SD crops. Realising a high photosynthetic potential while simultaneously and quickly initiating flowering and maturity is important for speed breeding SD crops. Additionally, investigating favourable light quality treatments might be worthwhile to accelerate the life cycle of crops in a speed breeding system.

Use of LED lighting technology and light quality control

The use of light emission diodes (LED) in horticulture was considered a breakthrough regarding plant productivity (Massa et al. 2008, Morrow 2008) as well as energy and maintenance costs (Schratz et al. 2016). In fact, the initial high purchase costs of LED lamps are compensated in the long run given their much longer lifespan and their reduced energy consumption per wattage, which still continues to improve (Schratz et al. 2016). The very narrow and specific emission spectra of LED compared to fluorescent lamps (Gupta et al. 2013) allow a very precise adjustment of light quality by including or excluding certain wavelengths within the photosynthetic range to and from the emission spectrum. Although Ghosh et al. (2018) published their light quality conditions in their speed breeding setup, they did not experiment with different light quality protocols. It was shown that in crops like lettuce, spinach, radish and *Brassica* species different wavelength treatments can have significant effects on the development and the constitution of the plants (Yorio et al. 2003, Olle and Viršile 2013).

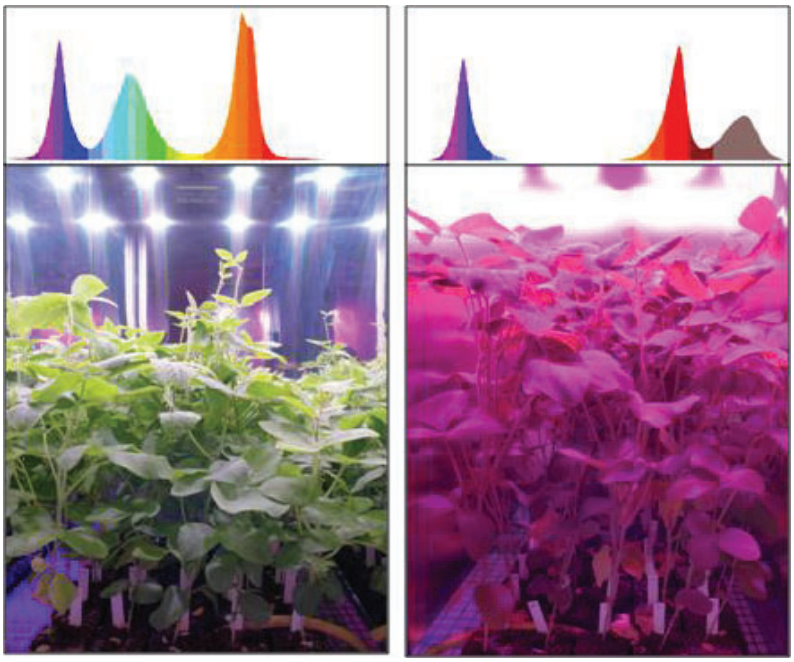

Figure 3 Soybeans in LED climate chambers exposed to two different light treatments. Emission spectra of the light treatments are depicted on top.

There seems to be little crop-specific knowledge about lighting requirements both in horticultural plant production but also for speed breeding purposes. Crop-specific adjustments of light quality protocols for speed breeding might have a significant impact on the length of the breeding cycle and thereby carry potential to increase genetic gain from selection.

Use of citizen science for plant breeding

In 2016 the University of Hohenheim and Taifun-Tofu GmbH launched the citizen science project “1000 Gärten”. Citizen science includes a broad public - mostly on a voluntary basis - in performing experiments and acquiring data. Thereby the public actively assists in research projects (Silvertown 2009). A total of 2492 participants volunteered in the project ‘1000 Gärten’ to grow 1710 soybean breeding lines and 20 market varieties in their private gardens (10 lines plus 2 check varieties in each garden). They were provided with the seed material, work instructions and time tables and ultimately asked to evaluate 16 phenotypic traits throughout the growing season. The data was collected from an online database. While the concept of citizen science has been used in ecological research in the past (Magurran et al. 2010, Silvertown et al. 2011, Worthington et al. 2012) it is a novel tool for agricultural studies and has not been performed on such a scale before. The ‘1000 Gärten’ study far exceeded the number of trial locations typically featured in agricultural multi-environment trials. One focus of the study was to shed light on the adaptation of soybean lines in terms of maturity to specific geographic regions within Germany. It furthermore aimed to investigate genetic components of soybean underlying adaptation to northern growing areas and subsequently to evaluate the usefulness of the genotypes for breeding purposes. Publically available weather data from the ZIP-code area of the gardens were included into the analyses in order to deduce information about temperature requirements and photoperiod sensitivity of the soybean lines. Finally, a high number of laypersons were given the opportunity to grow soybeans in their own gardens. This enabled them to observe the plants first hand, challenging the rather negative public image of soybeans due to deforestation and growing practices especially in the Americas. This could trigger a broader and more nuanced discussion about the crop. The results of the study might help to draw conclusions about adaptation to CE climate conditions, identify mega environments and assess yield stability across many environments in Germany and CE.

Figure 4 Impressions from selected participants of the citizen science project „1000 Gärten“, 2016

Objectives of this study

The overall aim of this thesis was to promote the adaptation of soybean as an agriculturally valuable crop in Central Europe. Specifically three different approaches were evaluated that can also generally be used as tools for plant breeding:

- Elucidate the genetic architecture of soybean tolerance to chilling stress during flowering and pod formation towards genomics-assisted breeding
- Adapt the concept of speed breeding to soybeans (and SD crops in general) by means of LED-lighting systems under controlled climate conditions in order to accelerate the generation cycle and increase genetic gain
- Evaluate the potential of the citizen science approach as an additional element for agricultural research and plant breeding.

Publication 1

Cold stress tolerance of soybeans during flowering: QTL mapping and efficient selection strategies under controlled conditions

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Abstract

Plant breeding can help adapting soybean cultivars to grow in colder areas of the world because chilling stress during flowering leads to shedding of flowers and pods and thus ultimately yield loss. Natural tolerance towards these stresses can be evaluated under artificially controlled conditions in climate chambers. However, the trait cold tolerance is laborious to assess and given the limited space in controlled chambers, an optimal allocation of resources is required. 35 soybean cultivars (maturity group: early (MG00) and very early (MG000)) as well as a biparental population consisting of 103 recombinant inbred lines (RILs) were observed for their stress tolerance towards cold during flowering. High heritability values were calculated for number of pods independent of the treatment (control vs. cold conditions), however correlation of pod number between these artificial environments was low. Based on the data at hand we simulated scenarios differing for number of genotypes and replications. From calculating response to selection, we concluded that available resources in climate control chambers should be given to number of RILs or genotypes rather than replicates. The phenotypic and genotypic data revealed quantitative trait loci (QTL) on three chromosomes (7,11 and 13) for pod number of which only the QTL on chromosome 11 was found to be specific for pod number during cold stress. Additionally, a genomic prediction approach using 4 different test set sizes and 4 prediction models underlined that genomic prediction is a considerable tool in plant breeding, even more so if already detected QTL can be built into the model as fixed effects.

Publication 2

Speed breeding short-day crops by LED-controlled light schemes

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ORIGINAL ARTICLE

Speed breeding short-day crops by LED-controlled light schemes

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Abstract

Key message **A simple and rapid speed breeding system was developed for short-day crops that enables up to five generations per year using LED lighting systems that allow very specific adjustments regarding light intensity and quality.**

Abstract Plant breeding is a key element for future agricultural production that needs to cope with a growing human population and climate change. However, the process of developing suitable cultivars is time-consuming, not least because of the long generation times of crops. Recently, speed breeding has been introduced for long-day crops, but a similar protocol for short-day crops is lacking to date. In this study, we present a speed breeding protocol based on light-emitting diodes (LEDs) that allow to modify light quality, and exemplarily demonstrate its effectiveness for the short-day crops soybean (*Glycine max*), rice (*Oryza sativa*) and amaranth (*Amaranthus* spp.). Adjusting the photoperiod to 10 h and using a blue-light enriched, far-red-deprived light spectrum facilitated the growth of short and sturdy soybean plants that flowered ~ 23 days after sowing and matured within 77 days, thus allowing up to five generations per year. In rice and amaranth, flowering was achieved ~ 60 and ~ 35 days after sowing, respectively. Interestingly, the use of far-red light advanced flowering by 10 and 20 days in some amaranth and rice genotypes, respectively, but had no impact on flowering in soybeans, highlighting the importance of light quality for speed breeding protocols. Taken together, our short-day crops' speed breeding protocol enables several generations per year using crop-specific LED-based lighting regimes, without the need of tissue culture tools such as embryo rescue. Moreover, this approach can be readily applied to a multi-storey 96-cell tray-based system to integrate speed breeding with genomics, toward a higher improvement rate in breeding.

Introduction

Key message: A simple and rapid speed breeding system was developed for short-day crops that enables up to five generations per year using LED lighting systems that allow very specific adjustments regarding light intensity and quality.

Conventional breeding of new and improved cultivars can take up to 12 years for annual crops from the point of crossing parental material until commercial release of novel cultivars. It is possible to significantly shorten this long and tedious process, for example by the use of winter nurseries, utilization of the doubled haploid technique (Thomas and Forster 2003) or the use of genetic engineering or genome editing (Araki and Ishii 2015). However, these approaches have severe disadvantages: winter nurseries are often expensive, logistically complicated to manage and do not guarantee successful seed production; doubled haploids are not available for most crops and often require highly qualified personnel and financial resources; and transgenic or genome-edited crops are often not a viable option because of political legislation or societal skepticism. Speed breeding was recently proposed by Watson et al. (2018) as an alternative to facilitate the simple and fast generation of new crop cultivars. The proposed speed breeding protocol reduces the generation time of long-day crops by an extension of the photoperiod to almost full day and harvest of immature seeds. However, this approach is limited to long-day crops and cannot be applied to short-day and photoperiod-sensitive crops, such as the globally important soybean and rice, because the prolonged photoperiod will prevent their

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flowering. Furthermore, the proposed protocol does not consider light quality to optimize the speed breeding procedure. Here, we present a light-emitting diode (LED)-based speed breeding protocol for short-day crops, that in addition to photoperiod also highlights the effect of light quality parameters for a practicable and high-throughput rapid single seed descent (rSSD) system (Fig. 1a, b).

Material and methods

Plant material and growth conditions

For the soybean experiments, we used seven commercial European soybean cultivars and one soybean line from the US Department of Agriculture Gene Bank. The panel of genotypes represents a broad range of maturity groups, from very early, photoperiod-insensitive cultivars that can be grown in Central Europe (maturity group G000), to late and photoperiod-sensitive subtropical lines (maturity group G5). Table S1 gives an overview about this panel, including cultivar name, maturity group and allelic constitution at the

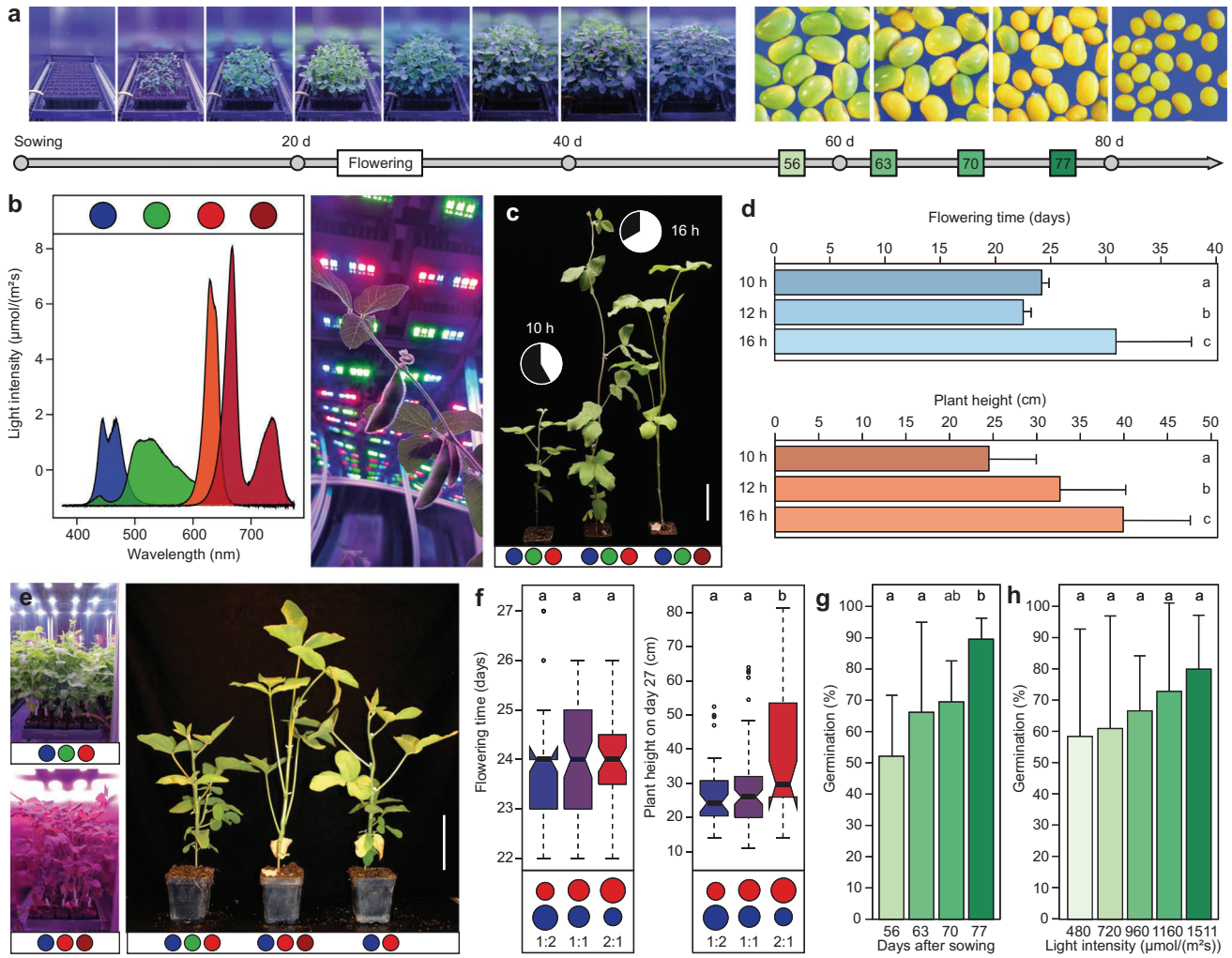

Fig. 1 Development of a speed breeding protocol for soybean. **a** Schematic overview of the steps to be optimized: the time to flowering and to maturity. **b** LED light spectrum with four separately controllable channels and impression from inside a LED speed breeding box. **c** Soybean grown under short-day and long-day conditions. Scale bar = 10 cm. **d** Bar plots showing the effects of day length on flowering time and plant height under blue, green and red light conditions. **e** Effect of red and far-red light on plant morphology. Scale bar = 10 cm. **f** Flowering time and plant height dependent on different ratios between red and blue light. **g** Germination rate of soybean seeds harvested at different time points. **h** Effect of light intensity on germination rate at 63 days after sowing

major maturity loci *E1*, *E2*, *E3* and *E4*. The rice and amaranth flowering time experiments included seven subtropical and tropical rice lines and seven amaranth lines with varying photoperiodism, the names of which can be found in Tables S2 and S3.

A mixture of two parts turf/wood fiber/clay granule substrate (NPKMgS 180/210/360/100/150 mg/l, Substrate 5, Klasmann-Deilmann GmbH) and one part sterilized soil substrate (compost soil/sand 3:1) was used as growth medium for all experiments. Substrate mixture for soybean experiments was inoculated with 'Soya Bean Inoculum' from Legume Technology Ltd. (Nottinghamshire) to allow formation of rhizobia. Plants were grown in pots measuring 7 cm × 7 cm × 8 cm (40 pots per tray) and for a few experiments in 96-cell plates (pot size 2 cm × 2 cm × 8 cm each). Watering was adjusted according to the plants' developmental status which in the soybean experiments required a weekly adaptation until pod onset. After pod onset, the water consumption of the plants remained constant at ~ 2 l/day on a tray with 40 plants. We watered the plants from below into the trays once every day with an automatic watering system.

All experiments were performed within a climate-controlled chamber. The temperature was set to 28 °C day and night. Humidity ranged from 80% to almost 100%, depending on the number of experiments in the climate chamber and watering regime.

Speed breeding boxes

Inside the climate chamber, we installed 12 different speed breeding boxes (80 cm × 60 cm × 120 cm) that allowed a parallel testing of different settings for parameter optimization (Fig. S1). The boxes were set up with reflecting surfaces on the inside to ensure optimal light usage. The light sources in each speed breeding box were light-emitting diodes (LEDs), which have the advantageous ability to emit very wavelength-specific light spectra as opposed to incandescent lamps. Several different LED systems were explored for all our 66 experiments using soybean, rice and amaranth:

LED modules: Ecotune, Daypro and Beaglebone

CompLED (COMPLED Solutions GmbH, Dresden, Germany) designed these LED modules. Hitz et al. (2019a, b) described the technical specifics of these speed breeding boxes in detail. Different types of LEDs were combined in four to six channels in each growth module. These channels are independently accessible via a user interface, which allows to regulate each channel in its intensity of light emission and the time of light exposure. The light emission intensities, and the spectra of each channel as well as all channels combined are shown for each module at 100% light intensity in Table S4 and Fig. S2 (1–3).

LED module: Relumity

Relumity (Relumity TTI-Technologie-Transfer-Initiative GmbH, Stuttgart, Germany) builds two LED modules of this type. Contrary to other manufacturers, Relumity uses a modular setup, meaning that LEDs of the same wavelength are spotted on separate circuit boards, making it easy to alter light regimes by installing different circuit boards. The light recipe in this chamber type is controlled manually, the day length automatically by timer. The light emission intensities, and the spectra of each channel and all channels combined are shown at 100% light intensity in Table S4 and Fig. S2 (4 and 5).

LED module: Blue panel and blue rail

Growking (Growking.de, Leinfelden-Echterdingen, Germany) manufactured a third LED module in two different light intensities. Light regimes and intensities were not adjustable after delivery, but both LED systems were designed according to our requests. The light emission intensities and spectra of the Growking Blue Panel and Growking Blue Rail are shown in Table S4 and Fig. S2.

Rice and amaranth experiments

For the experiments with rice and amaranth, we used the following light protocol in the '*ecotune*' LED growth chambers: 556 and 574 µmol/(m^2s) light intensity, respectively, 10-h light and 14-h night, near-red light recipe with channels 1 + 2 + 3 and far-red light recipe with channels 1 + 2 + 4 (Table S4).

Phenotyping flowering and plant height

The soybean experiments were performed with five replicates of each cultivar. The 40 pots were completely randomized on each tray. For the experiments with staggered harvest dates, we used the three cultivars 'ES Senator', 'Josefine' and 'Nogoshi' and divided each tray into four compartments with 10 plants each (minimum three replicates per genotype per compartment) as shown exemplary in Fig. S4. We examined the plants' flowering every day after initiation of the budding phase. Flowering time was noted as days after planting upon appearance of the first petals on each plant. Since we aimed to present a rapid SSD protocol, we decided to stop every experiment in which a genotype exceeded a flowering time of 40 days. We measured plant height 20 and 27 days after sowing as the distance from the plant's shoot apical meristem to the soil. Some genotypes showed indeterminate growth under these conditions and were pruned on day 30 after sowing. An overview of the performed experiments, light quality and quantity conditions,

flowering and plant height per genotype and per treatment is shown in Table S5. We noted flowering time for rice when the first panicle emerged and for amaranth when the first pollen sacs were visible. Plant height of rice and amaranth was measured upon termination of flowering from the tip of the inflorescence to the soil. Tukey's honest significant difference (HSD) test was used to test pairwise comparisons and significant differences of means (Tukey 1977). Different letters on the top of box plots and bar plots indicate significant differences of the means according to Tukey's HSD test ($\alpha = 0.05$).

Harvesting

Harvesting dates (~ 63 and ~ 70 days) differed between the performed experiments in order to test different maturity periods. In all experiments, watering was ceased 5 days before harvesting date to accelerate the ripening process in seeds. In experiments with staggered harvest dates (55/56, 63, 70 and 77 days), we moved all plants that reached the end of the watering period into a smaller waterproof compartment tray in order to prevent those plants from being watered for 5 days (Fig. S3). On the harvest day, the pods of every genotype were bulked. Afterward, a drying treatment was applied for 24 h at 37 °C to facilitate manual cleanup of the seeds from their hulls. Table S6 shows a list of all experiments that were carried out until the harvest of the plants to assess their germination ability.

Germination experiments

Experiments concerning germination tests started with the following conditions: blue, green and red (630 nm) lights under short-day conditions (10 h) with a light intensity of 556 µmol/m^2 s until day 35 after planting (Table S5; Exp. 29, 33, 36, 37, 40, 46 and 53), and were then transferred to different conditions until harvest (Table S6). Exceptions from this procedure were made in experiments 51, 55, 56 and 66 in which the plants were grown under the same conditions from germination to harvest (see corresponding Exp. 43, 48, 50 and 61 in Table S5). All seeds were treated with 0.05% thiram solution (Aatiram 65, Cheminova) for two minutes in order to reduce fungal growth during the germination tests. Germination took place on filter paper; under dark conditions, the temperature was set to 25 °C. Sterile water was used to keep the filter paper moist. Germinated seeds were counted on day 7. Missing radicle protrusion was considered as not germinated. Germination rate was calculated as the quotient of germinated seeds divided by the total number of used seeds in the experiment. In order to investigate the impact of gibberellin on the germination of seeds from 56-day-old soybean plants, we used the genotypes 'Merlin,' 'ES Senator,' 'Amphor' and 'Aires.' The plants were grown at a light intensity of 420 µmol/m^2 s without far-red light. Germination protocol was as described above, with one half of the laid-out seeds per genotype watered with ddH_20 and the other half with 0.1% (v/v) Gibb + -Solution (Gibb^{Plus}, Plantan). Germinated seeds were counted on day 4, 7 and 10 after the start of the experiment.

Results

In photoperiod-sensitive short-day crops, long-day conditions hinder the initiation of flowering. Hence, lighting protocols longer than 12 h can be expected to lead to delayed flowering, but on the other hand may enhance carbon accumulation and might therefore speed up seed production (Chatterton and Silvius 1979; Jensen and Veierskov 1998). In addition, it is known that phytochrome-deficient genotypes of rice, sorghum and soybean flower earlier under long-day conditions (Izawa et al. 2000; Childs et al. 1997; Tsubokura et al. 2013; Cober et al. 1996) and that far-red light promotes the transformation of active into inactive phytochromes (Carré et al. 2005). Focusing on soybean, we therefore first evaluated light regimes with an increased light period of ≥ 12 h, but combined with a low red to far-red ratio (< 700 nm: > 700 nm) to potentially achieve early flowering with an increased photosynthesis rate (Table S5). In order to enhance photosynthesis rate further during night conditions, blue light (450–490 nm) was enabled. Smith and Whitelam (1997), Childs et al. (1997) and Craig and Runkle (2013) suggested that far-red lighting leads to earlier flowering. In contrast to these previous findings, in our experiments neither far-red nor an additional blue light treatment at night could accelerate flowering of the soybean genotypes. In most cases, average flowering time surpassed four weeks and was highly heterogeneous among the soybean genotypes, showing the different responses to day length of cultivars from different maturity groups. Generally, time to flowering was hastened and the plants were shorter under ≤ 12 h day length protocols (Fig. 1c, d). As shorter day length led to faster flowering, short-day protocols with light exposures of 12, 10 or 8 h were examined, which reduced the average flowering time to 23.9 ± 1.8, 23.7 ± 1.4 or 24.0 ± 0.8 days after planting, respectively. Moreover, under such conditions all soybean genotypes flowered in a homogeneous fashion, allowing to use the system for early and late maturity groups in the same growing cycle (Fig. S4).

Next, several experiments were performed under a day length set to 10 h, to determine the optimal light regime that maintains early flowering while reducing plant height so that the plants would fit into multi-storey tray systems. Far-red light (> 700 nm) did not affect flowering time, but led to an unwanted plant morphology with severely elongated petioles and consequently lodging, as also reported by

Smith and Whitelam (1997), Franklin and Whitelam (2005) and Hitz et al. (2019a) (Fig. 1e). Hence, far-red lighting must be avoided to obtain sturdy soybean plants suitable for compact high-throughput systems. Avoiding far-red wavelengths, we evaluated the impact of different red/blue light ratios (Fig. 1f). Generally, a lower red/blue light ratio led to shorter and sturdier plants without affecting flowering time, but when using only blue light no further height reduction could be observed. Although green light (500–560 nm) did not greatly affect flowering time and plant height of soybean, visual evaluation of the plants (scoring of diseases, presence of pests) was highly improved by including green light into the lighting protocol (Fig. 1e). Thus, a multi-storey speed breeding protocol for soybean must avoid far-red light (> 700 nm) and should have a low red/blue light ratio, with green or cool white LEDs (4000 K) included for optimal visual observations.

Since lighting schemes with more than 12-h light are not suitable for short-day crops, but as enhanced photosynthesis rates may shorten cycle durations, we evaluated the impact of light intensity on flowering time and plant morphology for a 10-h lighting scheme. Increasing light intensity above 1000 µmol/m^2 s led to a ~ 2 days earlier flowering (21.8 ± 1.1 vs. 23.9 ± 1.1 for light intensity of 500–900 µmol/m^2 s) and shorter plant stature. These findings point to a further speed-up option by increasing light intensity, but these subtle changes will come with additional expenses due to higher energy costs and more expensive LED systems. Thus, for a well operating system a light intensity of ~ 500 µmol/m^2 s at 50 cm from the light source should suffice to achieve fast generation times on a moderate budget.

We next investigated whether this optimized light protocol elaborated for soybean would also enable speed breeding of other short-day crops, and ran flowering time experiments with rice and amaranth. In addition, we again assessed the impact of far-red light on flowering time and plant morphology. Contrary to soybean, rice flowered on average 7.9 days earlier under far-red light conditions than under far-red deprived lighting schemes (54.3 ± 7.7 vs. 62.2 ± 16.2 days) (Fig. 2a–c; Table S7). In fact, the majority of the genotypes

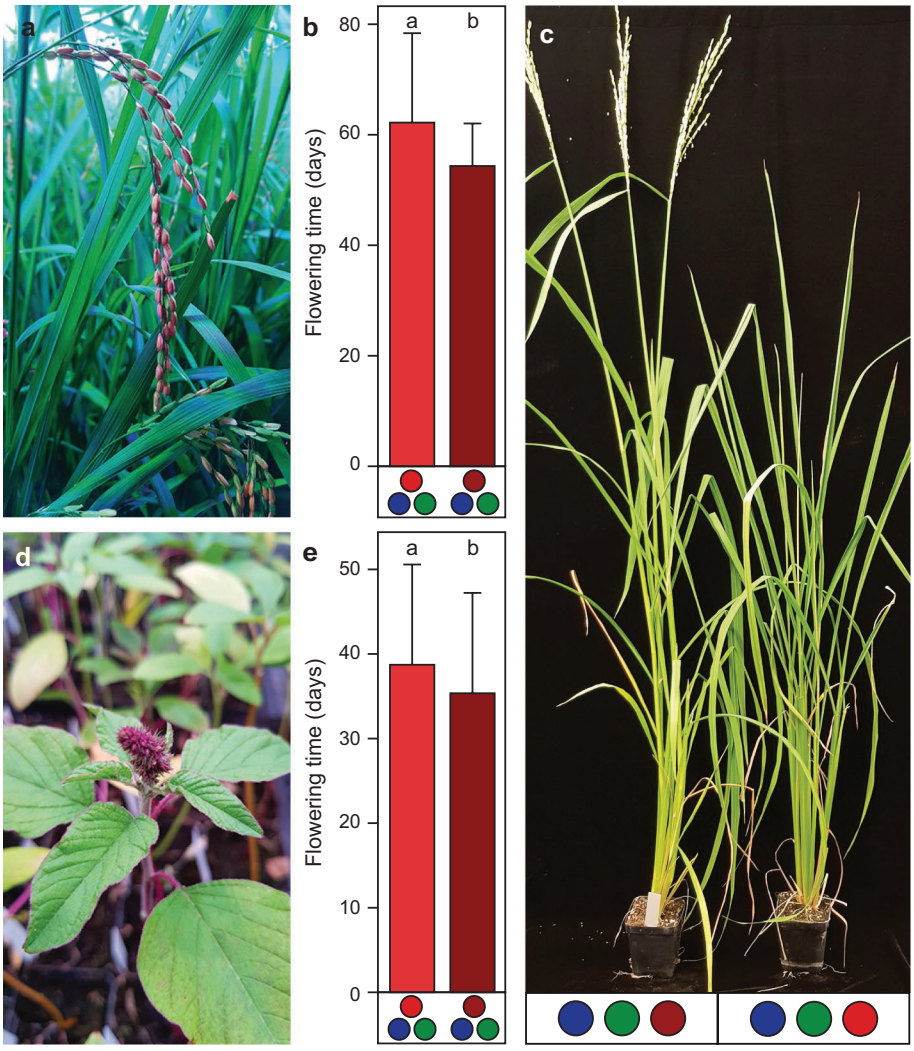

Fig. 2 Speed breeding rice and amaranth. **a** Rice and **d** amaranth flowering under speed breeding conditions. **b** Effect of red and far-red light on flowering time of rice. **e** Effect of red and far-red light on flowering time of amaranth. **c** Rice genotype 'Nerica L-19' on day 70 after sowing under far-red and red light conditions

flowered almost equally fast under both light conditions. However, two of the genotypes which took the longest to reach inflorescence stage ('Primavera' and 'Nerica L-19′) flowered 20 days earlier under far-red light compared to the near-red light protocol. A similar picture was obtained for amaranth that flowered on average ~ 3 days earlier under far-red light (35.35 ± 11.87 vs. 38.71 ± 11.85) (Fig. 2d, e; Table S8). As observed for rice, there was a strong genotypic dependency on light quality (Fig. S5, Table S7, S8). Similar to soybean, plant height of rice and amaranth increased under far-red conditions, but did not lead to instable plant stands as observed for soybean. Generally, these findings show that speed breeding protocols cannot be readily interchanged from one short-day crop to another and especially that light quality must be considered for an optimized genotype-independent speed breeding system.

Achieving fast flowering is the first step in setting up speed breeding protocols. Once fast flowering is reached, fast maturity or germination of immature seeds needs to be accomplished. Aiming at a two-month seed-to-seed protocol for soybean, we evaluated germination rates at staggered harvest time points (day 56, 63, 70 and 77). Mean germination increased from ~ 50% on day 56 to ~ 90% on day 77, and likewise the homogeneity of germination across the genotypes increased (Fig. 1g; Fig. S6). Although 77 days after sowing yielded very good germination rates, we tested whether increased light intensities could further improve germination rate at earlier time points, by either using stronger light sources or extending lighting duration after flowering onset. We observed a general increase in germination rate with lighting intensity, i.e., using 10-h day lengths from ~ 60% at 480 µmol/m^2 s to ~ 80% at 1511 µmol/m^2 s at 63 days after sowing (Fig. 1h). To further speed up the maturation process, day length was extended from 10 to 18 h on day 35 after pod onset was initiated. Although germination rate increased in the 18-h treatment by ~ 8% at 63 days after sowing, germination rate was still < 70% and highly variable among genotypes. Next, we increased the light intensity to 1160 µmol/m^2 s and aimed at a harvest time on day 63 after sowing with three different light regimes. Germination was slightly increased when including green light (78%) as compared to the red and blue (71%) or the far-red lighting schemes (62%). Although acceptable mean germination rates were achieved, there were still large genotypic differences as compared to seeds that matured until day 70. Furthermore, such high light intensities again come with the more expensive LED light sources and energy costs. Given that some genotypes showed optimal germination results even in the 63-day-long tests using lower light intensities, it is possible to reduce the generation time of certain populations or breeding material. However, since synchronization of maturity could not be guaranteed across genotypes, we conclude that a longer maturation time to ~ 75 days is necessary for a speed breeding system that can operate genotype independently and without the need of time- and work-intensive methods such as the growth on sterile medium or embryo rescue in order to facilitate germination of unripe seeds. We also investigated whether a gibberellin treatment was able to further improve the germination of unripe seeds as suggested by Hickey et al. (2019). Soybean seeds harvested from 56-day-old plants that were treated with Gibb + solution increased their germination on average by 7% as opposed to the water control (Fig. S7). However, the germination was generally low at that early stage, a wide variation among genotypes was observed, and the difference between the treatments was not significant at any time point. More importantly, the Gibb + treatment had the unfortunate effect of elongating the hypo- and epicotyls of the germinated soybean seedlings, which is a highly counterproductive attribute keeping in mind a multi-storey growth chamber with limited space.

Although we established the protocol with larger pots, we also transferred it to 96-cell plates, with pot sizes of 75cm^3, allowing ~ 750 plants per cubic meter of space (Fig. S8). Soybean growth and flowering time were comparable to the described protocol, with plant height reaching ~ 34 cm on day 28 and flowering at ~ 24 days after planting at a light intensity of ~ 1000 µmol/m^2 s, and a seed-to-seed turnover of ~ 75 days. Seed number per plant was reduced in the 96-cell system, but with on average more than five seeds per plant sufficient for an rSSD system. Having a 96-cell-based system for plant growth allows the speed breeding protocol to be readily integrated with genotyping systems that are all based on 96-well microtiter plates. This will allow routine single marker and genomic selection approaches during the speed breeding process and reduce the error rate due to the full conformity between growth and DNA analysis system.

Discussion

Speed breeding is a powerful tool for plant breeding and plant genetic research. Our results for the three short-day crops, soybean, rice and amaranth, highlight the need for crop-specific lighting schemes that can speed up the time to flowering and maturity and might be utilized to improve germination. For soybean, we developed a protocol that allows up to five generations per year, as compared to one generation on the field or 2–3 generations if winter nurseries are used (Fig. 3). Increasing the speed of our protocol by raising the CO_2 level can be considered (Nagatoshi and Fujita 2018), but might be only advantageous if a higher photosynthesis rate may also be guaranteed through more intense lighting. The additional speed should be weighed against the extra costs of the system setup and operational costs (e.g., energy).

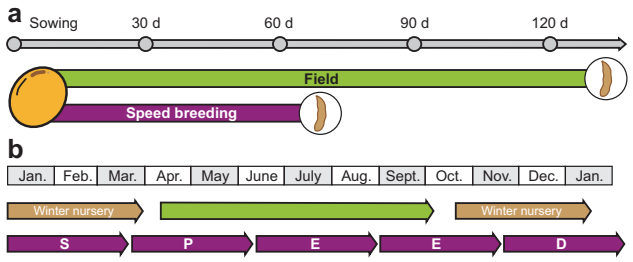

Fig. 3 Speed breeding short-day crops. **a** Time required for one soybean generation under field conditions in central Europe compared to speed breeding. **b** In contrast to the field, where only one or with winter nursery 2–3 generations can be achieved per year, speed breeding enables five generations per year

In general, speed breeding enables the introgression of monogenic traits that are easily scored within the climate chamber, but also allows crosses between genotypes from different maturity groups, which may widen the genetic variation of the breeding material and hence enhances response to selection. By obtaining up to five generations per year in a speed breeding system will lead to an approximately doubled annual genetic gain as compared to a fast breeding program which uses winter nurseries. Furthermore, tools such as marker-assisted or genomic selection can easily be incorporated in a speed breeding system, since logistical hurdles, which may arise in winter nurseries, are circumvented.

The fast generation of homozygous lines not only allows to speed up workflows in practical plant breeding but also for research purposes. Owing to the specificity of the LEDs, this system can also be used to dissect the interaction of specific wavelengths and the plant's physiological responses. Although our presented light protocol aims at researchers and breeders, companies working on and with indoor farming systems will profit from crop-specific research on light regimes like this one, too. Adjusted and smart lighting systems will play a pivotal role in urban farm production to meet future needs of the growing urban population that increasingly values short transportation routes, eco-friendly production and fresh food.

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Author contribution statement FJ and WLL designed the studies and planned the experiments. VH provided the plant material for the soybean experiments. FJ performed the experiments and analyzed the data. All authors wrote and edited the manuscript.

Compliance with ethical standards

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

Ethical standard The authors declare that the experiments comply with the current laws of Germany.

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Publication 3

The soybean experiment ‘1000 Gardens’: a case study of citizen science for research, education, and beyond

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ORIGINAL ARTICLE

The soybean experiment '1000 Gardens': a case study of citizen science for research, education, and beyond

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Abstract

Key message **Citizen science, an approach that includes normal citizens in scientific research, holds great potential also for plant sciences and breeding and can be a powerful research tool to complement traditional approaches.**

Abstract Citizen science is an approach that includes normal citizens in scientific research, but has so far not been exploited by the various disciplines in plant sciences. Moreover, global threats challenge human well-being and science can provide solutions, but needs to leave the ivory tower in the mind of the broader public. In 2016, we performed the '1000 Gardens—the soybean experiment' citizen science project, that aimed at finding citizens in Germany who would grow soybean lines in their own gardens and evaluate them for a range of traits related to adaptation and agronomic performance. Here, we describe details of this project, i.e. the recruitment, performance, and compliance of the citizen scientists. A total of 2492 citizen scientists volunteered for the project, but through the high media coverage a much broader audience than just the participants was reached. Our 1000 Gardens project was successful in collecting a scientifically unique data set with heritabilities ranging up to 0.60 for maturity date or 0.69 for plant height. Our results suggest that the citizen science approach holds great potential also for plant sciences and can be a powerful research tool to complement traditional approaches. Our project was also successful in raising public awareness about the importance of plant breeding and in communicating key messages on the manifold benefits of legumes for a sustainable agriculture to a broader public. Thus, citizen science appears as a promising avenue to demonstrate the value of breeding and science to the general public by including normal citizens in scientific research.

Introduction

Citizen science is an approach that at least in part involves citizens as highly respected partners in research (Silvertown 2009). The idea itself has a long tradition, as for decades the help of such non-professionals has proven to be an invaluable and extremely powerful tool for ornithology to survey population numbers and spread (Magurran et al. 2010). The earliest what we now call citizen science projects, is probably the Christmas Bird Count by the National Audubon Society that has been running in the USA since 1900. More recently, a few other disciplines have also adopted this approach, and it has, for example, been successfully used in ecology and environmental sciences to monitor invasive species or in astronomy, where it has recently led to the discovery of a novel brown dwarf (Silvertown 2009; Magurran et al. 2010; Worthington et al. 2012; Kuchner et al. 2017). Worthington et al. (2012) employed the citizen science approach in the Evolution MegaLab project that surveyed shell polymorphism in two banded snails across Europe with the aim to compare these data with historical records to detect evolutionary change.

Generally, citizen science projects are designed either to generate scientific data for research or for the educational benefit of the amateur volunteers, ideally for both. Citizen science projects could, however, have a far greater potential than purely as a research tool or for the education of the participants. An exciting project may serve as a vehicle to transport a range of messages beyond the particular research

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question of the project. It could provide a platform for the participants to share their experiences and give them the feeling of a collective action, which could spread and grow through the participants as multipliers. Most importantly, appropriately designed projects could arouse moral emotions, i.e. make even complex topics emotionally accessible and thus open up the general public to scientific arguments and their consequences.

One such challenge that requires global change is the establishment of sustainable agriculture. The United Nations declared 2016 as the International Year of Pulses (grain legumes), under the slogan 'nutritious seeds for a sustainable future' (www.fao.org/pulses-2016). In contrast to cereals, grain legumes are capable of symbiotic atmospheric nitrogen fixation and generally have several desirable attributes, as they enhance biodiversity in our agro-ecosystems, contribute to soil fertility, and benefit both humans and livestock due to their advantageous nutritional characteristics (Foyer et al. 2016). A reduction in meat consumption by shifting to grain legume products could substantially reduce the carbon footprint caused by the production of protein for human consumption. Soybean is the most important leguminous crop worldwide, but Europe is currently heavily dependent on soybean imports. Soybean was never a major crop in Germany, and its cultivation has only recently seen a revival with a strong relative increase in soybean acreage. This was mainly driven by political efforts to reduce the high dependency on plant protein imports, but also by the desire of consumers for genetically modified (GM)-free, regional products. While the majority of the domestic soybean production is used for animal feed, its use for human consumption, especially through tofu products, has seen a tremendous increase, owing to strong consumer trends for vegetarian or vegan food, as well as for novel and healthy products that diversify our food basket. Compared to the traditionally cultivated crops, however, soybean still has a rather negative public image, mainly as the broader public associates it with being genetically modified, grown in large monocultures, and contributing to the deforestation in South America, Asia, and elsewhere.

Except for ecological studies, the citizen science approach is not yet recognized as a research tool for plant sciences. In 2016, the University of Hohenheim and the Taifun-Tofu GmbH performed the '1000 Gardens—the soybean experiment' citizen science project, that aimed at finding citizens who would grow and evaluate soybean lines in their own gardens. The objectives of the project were to (1) collect data for scientific analyses such as the genetics underlying the adaptation of soybean to more northern latitudes and (2) improve the public image of soybeans and highlighting the manifold advantages of soybeans and legumes in general for a sustainable (regional) agriculture as well as the health-associated advantages of grain legume-rich diets. While normal plant breeding trials are performed at only a few locations, this approach has enabled data to be collected on the performance of soybean genotypes at an unprecedented number of locations throughout Germany. This allows the identification of genotypes that are adapted to more northern latitudes or regions where soybean is not yet widespread. Here, we describe how we planned and executed the 1000 Gardens project and make suggestions on how to improve future citizen science projects, as we believe that this approach has great potential also for various disciplines in plant sciences, for research, breeding, education, and beyond.

Materials and methods

Recruitment of citizen scientists

Prior to the start of the project, a website was designed to provide background information on soybean, the history of its cultivation, soybean for human consumption, as well as its positive effects for a sustainable agriculture and an optimal human diet (www.1000gaerten.de; Fig. 1a, b). The website provided guidance for participants on how to conduct the experiment and other sections including a blog for the citizen scientists to share their experiences and results. The project started in early 2016 with press releases and advertisements placed in several magazines on garden- or food-related topics, as well as on Taifun-Tofu products, accompanied by the launch of the website. Registration was open until the end of February 2016.

Plant material

The soybean panel used in this study is comprised of 1710 breeding lines that can all be classified as early maturing material of maturity groups (MG) 000 and MG 00. The 1710 lines are derived from crosses among 20 parental lines of the same two maturity groups. The progeny from each cross were taken to the F_6 generation by single-seed descent and were then continued as bulks. The parental combinations and the number of $F_{6:7}$–$F_{6:9}$ progenies in each cross are shown in Table S1. Some participants were sent 10 different breeding lines for evaluation (Experiment 1), and in the conditions of participation it was specified that the intellectual property rights of the breeding lines remained with the organizers of the study. The remaining participants were sent ten varieties (Experiment 2): 'Alexa', 'Abelina', 'Merlin', 'Regina' and 'Sunrise' of MG 000, 'Lenka', 'Primus', 'Korus', 'Shouna' and 'Solena' of MG 00. In addition, each participant got the two check varieties 'Taifun3' and 'Adsoy', where 'Adsoy' is an extremely early maturing genotype of MG 000 that might

Fig. 1 The soybean '1000 Gardens—the soybean experiment' citizen science project ('Die Sojapflanze', Soybean; 'Das Experiment', The Experiment; 'Ergebnisse', Results; 'Gärtnerblog', Gardeners' Blog; 'Wer wir sind', Who we are). **a** The 1000 Gardens project website (www.1000gaerten.de) and **b** logo of the project. **c** The starter pack sent to the participating citizens contained twelve bags with 100 seeds each, a bag with inoculum for Rhizobacteria inoculation, labels to mark the rows in the garden, a card to score the greenness of the leaves, and instructions on when and how to sow the seeds and to score the target traits

even be classified as MG 0000 and 'Taifun3' is slightly later maturing and from MG 000.

Assessment of soybeans by the participants

The participants were sent instructions for the experiment, twelve bags with 100 seeds each for rows of 2 m length and a bag with inoculum for Rhizobacteria inoculation (Fig. 1c). Participants were asked to thin the germinated plants at a distance of 4 cm. Between rows a space of 0.5 m was recommended. The two common check cultivars were grown by each participant as the outermost rows, whereas the order of the ten breeding lines and cultivars followed the randomization.

Until harvest 16 morphological, physiological, and yield-related traits were listed for evaluation, including flowering, maturity, height, and pods per plant (Table 1). The participants entered the phenotypic data collected in their own gardens online into a database for scientific analysis.

Statistical analysis

Owing to the high number of participants, we performed two experiments. Experiment 1 included 1730 gardeners and was based on the 1710 breeding and their 20 parental lines, i.e. 1730 genotypes. It was laid out as an α-lattice design with ten replications, each replication with 173 incomplete blocks with a size of 10 plots. Here, each participant represented an incomplete block with 10 genotypes. Thus, the

Table 1 Description of the trait data

Nr.	Trait	Data type	Month[a]	Heritability Exp. 1[b]	Heritability Exp. 2[b]
1	Sowing date	Date	Apr., May		
2	Length of row	Metric (cm)	Apr., May		
3	Germination rate	Metric	May, June	0.40	0.99
4	Start of flowering	Date	June, July	0.28	0.63
5	Green value of the leaves	Nominal	July, Aug.		
6	Flower colour	Nominal	June, July		
7	End of flowering	Date	July, Aug.	0.42	0.80
8	Plant height	Metric (cm)	Aug., Sep.	0.69	0.98
9	Branching	Ordinal (w, m, s)[c]	July, Aug.	0.26	0.87
10	Distance lowest pod to ground	Metric (cm)	Aug., Sep.	0.55	0.96
11	Layers with pods	Metric	Aug., Sep.	0.41	0.94
12	Start yellowing of leaves	Date	Aug., Sep.	0.60	0.98
13	Lodging	Ordinal (1–9)	Aug., Sep.	0.32	0.92
14	Maturity date	Date	Aug., Sep.	0.60	0.96
15	Pods per plant	Metric	Aug., Sep.	0.28	0.84
16	Beans per 20 pods	Metric	Aug., Sep.	0.28	0.93
	Quality traits scored centrally on returned beans				
	Protein content	Metric (%)		0.81	0.99
	Oil content	Metric (%)		0.80	0.96

[a]Month when the trait is approximately scored

[b]Experiment 1 refers to the 1730 breeding lines and Experiment 2 to the 10 cultivars

[c]*w* weak, *m* medium, *s* strong

1730 soybean lines were randomly assigned to one of 173 gardeners in ten zones, i.e. the ten replications, two of which were combined into five regions following latitude (Fig. S1). The reason for this was to ensure an even distribution of each genotype in north–south direction, as photoperiod and thus latitude is an important determinant of soybean adaptation and maturity (Kurasch et al. 2017). For Experiment 1, the model was $y_{ijk} = \mu + g_i + r_j + b_{jk} + \varepsilon_{ijk}$, where y_{ijk} is the observed phenotypic value of the ith genotype in the kth block of the jth replication, μ the mean, g_i the effect of the ith genotype, r_j the effect of the jth replication, b_k the effect of the kth incomplete block nested within the jth replication (=garden of a participant), and ε_{ijk} the residual error.

The remaining 762 participants were part of Experiment 2, where each participant was sent the same ten cultivars and each garden represented a randomized complete block. The model for the analysis of Experiment 2 was $y_{ij} = \mu + g_i + b_j + \varepsilon_{ij}$, where y_{ij} is the observed phenotypic value of the ith cultivar in the jth block, μ the mean, g_i the effect of the ith cultivar, b_j the effect of the jth block and ε_{ij} the residual error.

Variance components were estimated in full random models, while best linear unbiased estimates (BLUEs) were estimated with genotype as fixed effect. Heritabilities were estimated following the suggestion by Piepho and Möhring (2007). All statistical analyses were performed using the statistical software R (R Development Core Team 2014) and ASReml-R 3.0 (Gilmour et al. 2009).

Results

Participation of citizen scientists

In total, 2492 citizen scientists volunteered to participate in the project (Fig. 2). The region in the south-west of Germany, particularly in the vicinity of the two cities Stuttgart and Freiburg, had more participants, also relative to their population, probably due to the two project partners being located there. Consequently, the initial press releases were more readily used for articles about the project in local newspapers and other media. This indicates that the number of participants in such an experiment can be controlled and thus increased through targeted media attention. Furthermore, Berlin also attracted a high number of participants compared to other major cities, probably due to its strong vegetarian and vegan scene. Obviously, people who are already interested in a topic are more open to participate, but the participants' comments indicated that there was no obvious bias towards a particular group, such as vegetarians or environmental activists. Rather, the driving force behind participation was that people found the experiment exciting

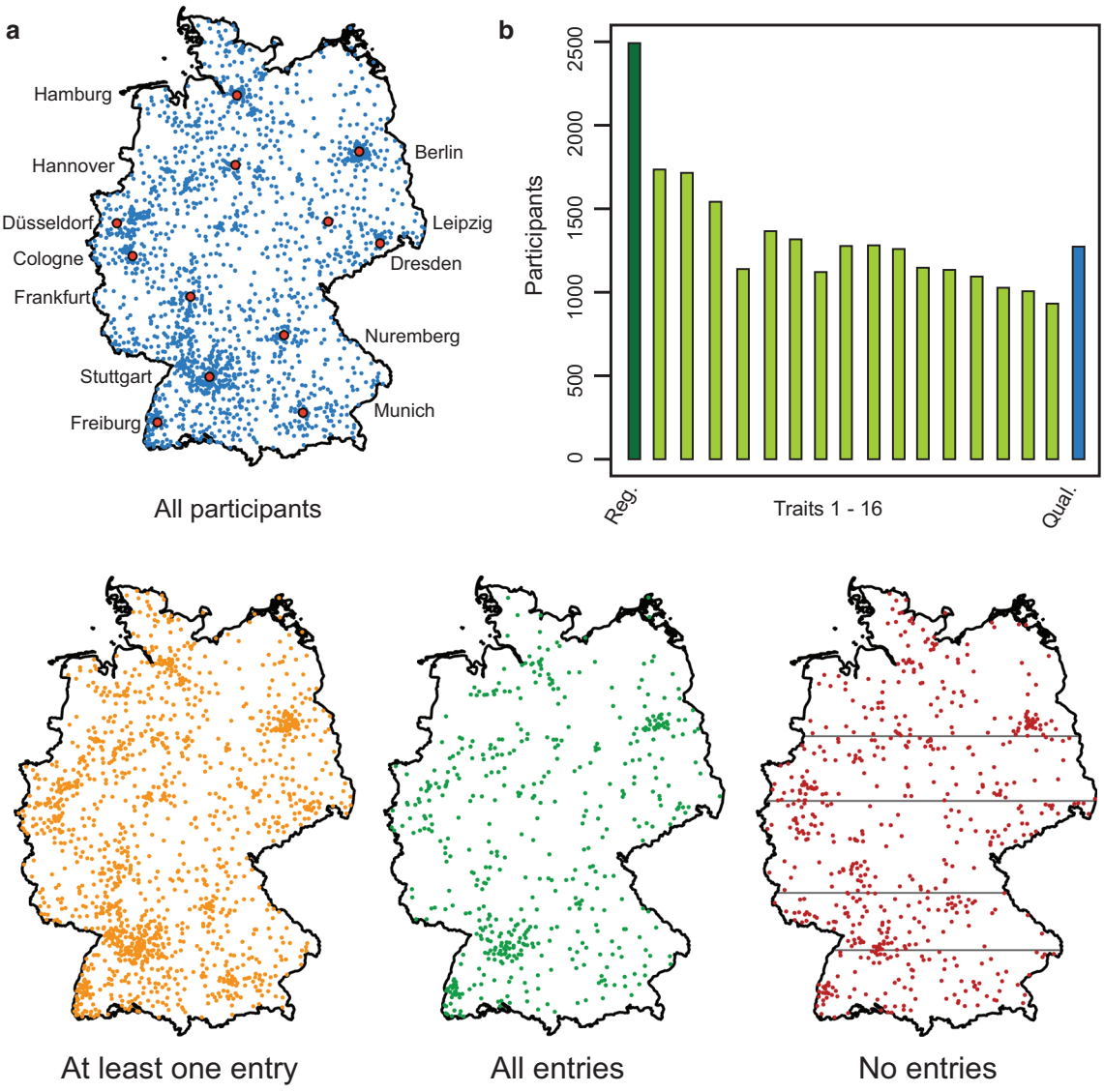

Fig. 2 Geographic distribution of the participants and trait assessment. **a** The maps show Germany with all 2492 participants, those who scored at least one trait and entered it online in the database ($n = 1790$), those who scored all traits ($n = 557$), as well as those without any entry in the online database ($n = 702$). The grey horizontal lines in the latter indicate the latitudinal regions considered during randomization. **b** Number of participants scoring each of the 16 traits (the numbering refers to Table 1) and sending samples back for quality analysis (Qual.)

and were attracted to take part to learn something and contribute to science. An interesting option for future citizen science projects is to include social scientists or psychologists in order to investigate in more detail the motivation of the participants throughout the project, their backgrounds, as well as the consequences possibly drawn by the citizens from participating in a project.

Quality of the data

The heritability of a trait expresses the fraction of the phenotypic variation that can be attributed to genotypic variation and can serve as a measure for the quality of the collected trait data. These heritabilities were high in the experiment with the twelve common cultivars but varied more strongly for the experiment based on the 1730 genotypes, ranging from 0.28 for the number of pods per plant to 0.69 for plant height (Table 1). The participants received instructions on how to assess the traits and were also reminded by emails around the time when the phenological stage to score each trait had arrived. However, the analysis also revealed that generally the quality of the data obtained by such an approach could be improved. The heritabilities of protein and oil content assessed on samples returned by the

participants were higher than those of traits evaluated by the participants themselves, indicating that not so much the locations increased the error, but the variable evaluation by the non-trained gardeners. An example for this is flower colour, which despite being highly heritable was seen and scored quite differently by the participants, who, for example, scored a purple petal with a small white spot as either purple or as mixed coloured. Thus, additional instructions focusing on visualization of the tasks, for example, by online video clips or apps for smartphones, appear promising to improve compliance and accuracy of measurements in citizen science studies. Nevertheless, the results, for example the high heritability with 0.60 for maturity as a basis to study adaptive mechanisms, demonstrate the value of the citizen science approach. This approach has yielded a unique set of data, and phenotypic information from such a large number of environments throughout Germany could not have been collected using traditional approaches.

Compliance of the citizen scientists

Of the initial 2492 gardeners who registered for the project and who were sent seeds, 30% ($n = 757$) never entered any data (Fig. 2). By comparison, Worthington et al. (2012) reported that for the Evolution MegaLab project 62% of the participants who registered did not submit a record. Thus, a certain loss of participants must be expected for this kind of approach, which must be taken into account when planning the project. In our study, this resulted in varying numbers of datapoints for each genotype, of which some were completely missing in some of the predefined latitudinal zones. This might have been prevented by a higher number of replications per genotype and zone. On the other hand, 1790 citizen scientists did collect data and entered them online. However, this number of gardeners entering data was further reduced during the project, sometimes due to adverse events like snails or hail destroying the plants, but also because the participants lost motivation and admitted that the required work was greater than they had expected (Fig. 2). The 16 trait assessments were designed to keep the participants motivated throughout the whole project (Fig. 3), and 57% ($n = 1027$) of the active gardeners recorded data until maturity, while 71% ($n = 1273$) even sent samples back for subsequent analysis of quality traits (Fig. 2).

A blog was established for the gardeners on the project website, which turned out to be frequently used by the participants (Fig. 4). By the end of 2016, there were 827 blog entries from 463 participants. The blog was used to share experience and results, but also stories beyond the experiment. The vast majority of gardeners who made blog posts gave a very positive feedback and indicated how much they enjoyed being part of the project and how much fun they had seeing soybeans grow in their gardens. It was thus an important instrument to create a sense of community for

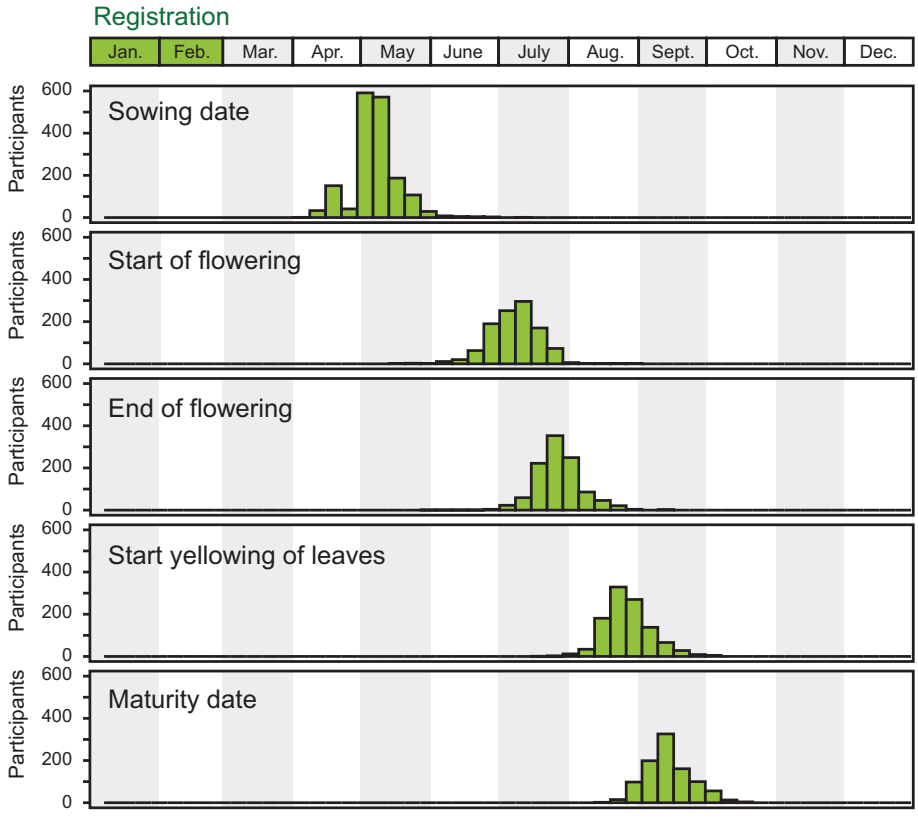

Fig. 3 Time course of trait assessments. Dates when the different traits were assessed by the participants in 2016, illustrating the distribution of trait assessments throughout the course of the project

Fig. 4 The citizen scientists at work. A collection of impressions of the citizen scientists at work, uploaded by themselves in the gardeners' blog

the participants, which probably also contributed to keeping them motivated. At the end of the project, a brochure was designed in which the scientific results and conclusions were shared with the citizen scientists (www.1000gaerten.de/aktivitaeten/ergebnisse).

Media attention of the project

From start to finish, the project attracted a great deal of media attention with articles and reports in print media, social media, radio, and TV. In print media, 461 articles appeared in 344 different newspapers and journals with a total circulation of almost 19 million, while online 235 articles were published (Fig. 5). In social media, the project was picked up 50 times, with a potential reach of about one million people. Moreover, 14 radio or TV reports covered the 1000 Gardens project. For all types of media, this attention covered the entire project duration. Although these numbers are estimates that are most likely to be incomplete, they do illustrate the great interest generated by the project in the

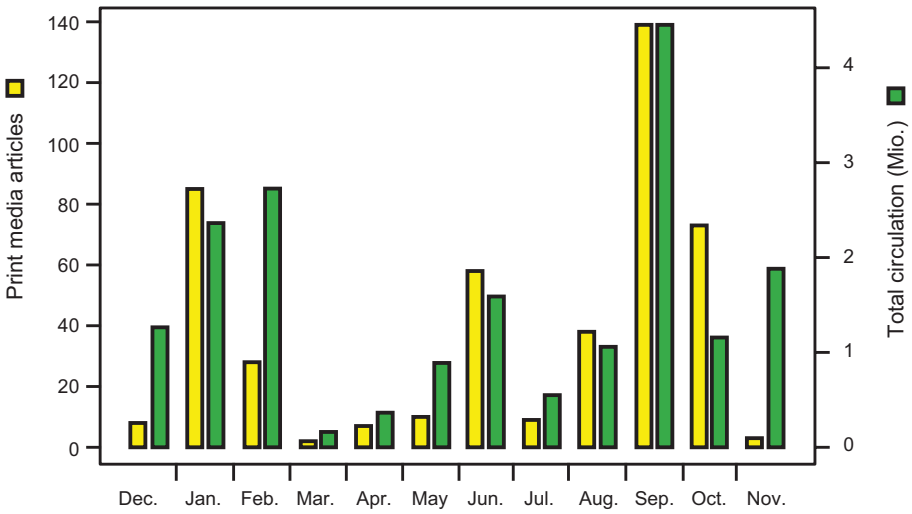

Fig. 5 Attention of the 1000 Gardens project in print media. Articles covering the 1000 Gardens citizen science project in print media and their total circulation. The period from December 2015 to November 2016 is shown

media, which has attracted a much broader public than just the participating citizen scientists.

Discussion

Citizen science complements the toolbox for plant science studies

Citizen science is not a new approach, but for a long time has been employed by only few disciplines, mainly to survey birds and other organisms. In recent years, citizen science has gone beyond ecology and environmental sciences and citizen scientists are now also participating in projects on monitoring water quality, climate change or addressing evolutionary questions (Silvertown 2009). This broader use of the citizen science approach was also facilitated by the availability of internet and online databases. However, most plant science disciplines have so far not yet applied this approach, because researchers were either unaware or did not know how to exploit it for their own research.

Here, we present details on the 1000 Gardens project. Beside the aim to improve the soybean public image by communicating key messages about the multiple benefits of soybean to the public, the project had the objective to collect scientific data, mainly related to the adaptation of soybean. Clearly, such a data set with trait data from hundreds of locations throughout Germany would not have been possible by traditional means. We will next combine these phenotypic data with molecular data in order to decipher the genetic architecture underlying adaptation in early maturing soybeans and to investigate the effect of the environment on quality traits. Moreover, the project had a strong focus on plant breeding, as the genotypes evaluated by the citizen scientists were breeding lines from an applied soybean breeding programme. Based on the results from this project, we were able to identify lines that are well adapted to different regions in Germany where soybeans are not usually grown. They also show a good agronomic performance and have high protein contents, an important criterion for soybean cultivars. Thus, after additional yield trials in the respective regions, these lines have a high potential to become registered soybean varieties, either for animal feed or even food grade and destined for human consumption, if they possess tofu quality. This also illustrates the great potential of this approach in breeding of regional varieties, i.e. varieties that are tailored to the specific growth conditions but also the requirements of the processors, marketers, consumers and policy makers in a certain region. Instead of citizens as in our study, this might be better achieved by cooperating with farmers, who are able to also assess the yield potential of selected breeding lines and who are familiar with the needs of the different actors of the production chain(s) in their region. Notably, the cooperation with either citizens or farmers in plant breeding can be regarded as a participatory breeding approach, which is a valuable tool in developing countries to establish local varieties that are accepted by the farmers (Atlin et al. 2001). As illustrated by our example, citizen science and participatory breeding can be congruent approaches and depending on the project, a range of different combinations is possible.

Consequently, the presented 1000 Gardens project was a citizen science project that can be assigned to the scientific categories crop genetics and plant breeding. Both are disciplines that one would not intuitively associate with citizen science. Our successful project therefore demonstrates that the citizen science approach can be a valuable research tool for many research areas, also in plant sciences. A prerequisite is that researchers are willing to consider this approach, which may mean to leave well-trodden paths and to tackle their research questions from a different angle in order to make it compatible with the citizen science approach.

Possible applications are, for example, the phenotypic evaluation of gene bank accessions under diverse climatic conditions in order to identify promising accessions for breeding, as well as to study adaptive mechanisms and genotype-by-environment interactions of crops or model species, thereby also demonstrating to the public the valuable work of gene banks in conserving this biodiversity. Particularly for vegetables the approach may prove valuable, as these are commonly grown by gardeners, who, in line with the basic idea of participatory breeding, might be involved when varieties with new characteristics or even entirely novel species are to be introduced into the market. Likewise, ornamentals appear predestined for the combined citizen science—participatory breeding approach, as they, too, are predominantly grown in gardens, on terraces or on balconies, by citizens who could score and also rate morphological and floral traits of breeding lines or yet not marketed species. The educational and scientific aspect in an ornamentals citizen science project might be the great insect die-off we are currently witnessing (Vogel 2017) and the identification of species or genotypes with a high attractiveness and nutritional quality for bees, butterflies, and other insects.

Future work should also address the question of the optimal experimental design for these kinds of studies, which, however, may vary depending on the particular set-up and the objectives of each project. Nevertheless, similar to the Mother and Baby trial design that meets the specific requirements of participatory breeding in developing countries by an iterative co-learning between farmers and researchers, the traditional experimental designs for plant breeding trials may not be the best for citizen science—participatory breeding studies as the one presented here (Bänziger and Diallo 2001; Atlin et al. 2002). As illustrated by the 1000 Gardens project, there is inevitably variation introduced by the mostly untrained participants. While this can be reduced by a better instruction and guidance of the participants, special attention should be paid to quality checks. An experimental design and statistical approaches that enable to assess the quality of the data of each participant would certainly be valuable in order to remove low-quality data and thereby improve the analysis of the entire data set and the conclusions drawn from it.

Our 1000 Gardens project was realized without a publicly funded project and a first grant proposal based on this experiment failed, as the reviewers were apparently unfamiliar with the concept of citizen science and were of the opinion that a traditional field trial would have been better. However, our results clearly show that even from a scientific point of view, this approach, which has not yet been used for most research areas, is attractive and can provide valuable and unique data sets. Thus, despite being novel and often without reference, funding bodies and organizations should open up to this kind of approach as a novel avenue to address scientific questions. Meanwhile, we were able to obtain funding for a second year of the 1000 Gardens project in 2018 through the German Federal Ministry of Education and Research (BMBF), which will allow to address additional research questions, for example genotype-by-year effects.

The value of science and its public perception

The March for Science that took place on 22 April 2017 in hundreds of cities around the world was an important step for science (Abbott et al. 2017), but its outreach and perpetuity may be limited as it did not emotionally involve a broader public. Important questions are, therefore, (1) how the public perception of global challenges and the willingness to act can be improved and (2) how the work of scientists and the value of research and scientific evidence as essential components to secure our health, environment, safety, society, economies; in short, our future well-being can be explained to the broader public.

Among the various reasons for the inaction to major threats is that they are distant in time and space. The polar bear and its melting arctic home or sinking islands at some far-off places are poor symbols for the consequences of global warming as they convey the message that the threat is someplace and sometime else but not right here and now. An important element to bridge that psychological distance is therefore the reduction of a scientifically complex problem of rather amorphous nature to something that people can understand and have access to, for example by growing it in their garden. Furthermore, active engagement as part of a larger group can provide strength to the individual, who might not act on its own, as they become part of a community effort and joint achievements. We suggest that citizen science projects hold the potential to assist us in achieving this.

Our 1000 Gardens project illustrates that the experiment performed by the citizen scientists does not have to directly tackle the complex topic or challenge, but rather must attract peoples' interest and be fascinating and entertaining to keep them motivated. It thereby allows to convey key messages that go beyond the actual experimental question. An important aspect is that the project provides a platform for discussion. Moreover, the projects may be designed to include children or young people as the future decision-makers. Similar to our study, the presented approach holds potential for a broad range of scientific topics or even to demonstrate the value of science itself.

Obviously, neither a single such project nor citizen science in general will stop climate change or save the world. However, citizen science can be a powerful scientific tool also for disciplines that so far have not considered this approach. In addition, such projects can be designed to educate people, but also to create a community feeling and

movement that, in combination with the media attention, can influence policy makers and thus result in change. Thus, in future scientists may not only be marching in the streets, but instead be figuratively marching into people's homes, thereby making the citizens scientists themselves.

Conclusions

Taken together, we hope that this report on the 1000 Gardens citizen science project provides input for scientists on how to carry out and optimize citizen science projects and encourage others to exploit this potentially very powerful research tool for their own research.

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Author contribution statement VH, MM, KB, and TW designed and performed the study. FJ and TW performed analyses. TW, WL, and VH wrote the manuscript.

Compliance with ethical standard

The authors declare that the experiments comply with the current laws of Germany.

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

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General Discussion

Molecular and genetic control of soybean cold stress tolerance during anthesis and pod formation

There is scientific consensus amongst climate researchers about global warming (Oreskes 2004, Cook et al. 2016) with earlier and warmer springs in CE (Xoplaki et al. 2005, Guiot and Corona 2010). As spring sets in earlier each decade in CE (Menzel et al. 2006) crop sowing dates have also been chosen earlier (Estrella et al. 2007). However, a trend towards a reduction in extreme weather events like cold spells or frost periods in spring-time Europe was not observed (Shongwe et al. 2007) and on top of that, these events are hardest to predict from March to May in Europe compared to the same time period on other continents (Monhart et al. 2018). This indicates the importance to breed for cold tolerant soybean cultivars in order to boost adaptation of soybeans to CE climate conditions despite the increased temperatures more favourable for this crop due to climate change.

This study confirmed the complex, quantitative nature of cold stress tolerance in soybean during flowering and pod formation with a certain amount of influence by major QTL. Those QTL were located on chromosomes 7, 11 and 13 in the biparental population Merlin × Sigalia under observation. This finding is in line with other studies that emphasise the complexity of cold tolerance but also elucidate molecular components which explain major parts of cellular mechanisms to cope with cold stress. Ikeda et al. (2009) for example reported a major QTL near the soybean Sat_162 marker. In another example, both soybean's C2H2-Type Zinc Finger Gene *GmZF1* (Yu et al. 2014) and *GmNEK1* (Pan et al. 2017) seem to promote drought and cold tolerance when cloned and expressed in *A. thaliana.* DREB2A transcription factor and Calmodulin-binding factor CAMTA1 are also discussed as molecular elements that are correlated with cold and drought tolerance while at the same time the underlying genetic complexity that enabled soybean's adaptation to colder areas was acknowledged (Yamasaki et al. 2016, Bandillo et al. 2017).

The detected QTL in our study on chromosomes 7, 11 and 13 may be of interest as targets for MAS. However, it must be noted that to date the detected QTL are neither confirmed in other populations, nor do they explain a large proportion of the genotypic variance for the yield component trait 'pod number at cold conditions'. Alleles that favour cold stress tolerance in the Merlin × Sigalia population originated from both parents although Merlin's ability to cope with cold stress was superior to Sigalia's. This, as well as the fact that we found individuals in the population that outperformed the better parent, supports a transgressive segregation for the trait 'pod number at cold temperatures'. The cold stress

QTL we discovered are not in proximity to any of the major maturity and photoperiod sensitivity genes *E1* (chromosome 6), *E3* (chromosome 19) or *E4* (chromosome 20), which is in contrast to the findings of Takahashi et al. (2005) or Funatsuki and Onishi (2009). Understandably a lot of breeding effort throughout the last decade has been put into pyramiding E-genes that influence the maturity of soybean in CE for photoperiodic reasons. The citizen science study “1000 Gärten” from 2016 revealed on the other hand that - within the early maturity soybean segment (MG00 and MG000) – the factor temperature weighed heavier than the factor day length regarding flowering and maturity even in northern areas of Germany. This raises the question whether further pyramiding of other maturity-influencing genes should remain the top priority or whether selection of cold-tolerant lines requires a stronger focus when breeding for soybeans adapted to environments of northern CE. Increasing the cold tolerance in early stages of soybean development in order to exploit a longer vegetation period by earlier sowing could be a promising approach. It would in turn help to exploit the greater yield potential of later maturing soybean genotypes with a longer vegetation period. A study by Littlejohns and Tanner (1976) concludes that cold tolerance in soybeans by means of germination and hypocotyl elongation at low temperature is maturity group independent. It suggests the possibility for breeders to combine both early maturity and increased cold tolerance in soybean’s early developmental stages.

Traits that underlie adaptation processes to specific environments are generally highly complex (Bandillo et al. 2017, Swarts et al. 2017). GS is a valuable instrument for plant breeding. It is able to consider these many undiscovered QTL that have small effects. Hence, genome-wide selection can play an important role in selecting for soybean lines that show better adaptation to colder locales. The decreasing costs of modern whole-genome sequencing techniques have brought up another potentially valuable tool to breed for cold-adapted crop varieties: the emerging field of landscape genomics (Rellstab et al. 2015, Bandillo et al. 2017, Haupt and Schmid 2020). It works to systematically detect loci that are hidden in genebank material or landraces from similar climatic regions. Detecting genetically valuable germplasm can assist in the development of core collections for plant breeding (Oliveira et al. 2010, Haupt and Schmid 2020). Using these core collections and their genetic potential to incorporate cold tolerance QTL from gene bank material into breeding programs may assist in the adaptation of crops to CE climatic conditions. Ultimately it may be an additional stepping stone that contributes to expand soybean’s agricultural growing sites to higher latitudes.

Potential of controlled environment phenotyping

According to the observed weak correlation of pod number between cold treatment and control temperature in the observed Merlin × Sigalia population of this study, direct selection

under stress conditions is necessary. Furthermore, this conclusion is supported by a high variance component for genotype-treatment interaction which decreases the chances to select the best cold tolerant genotypes under control temperature conditions. The similarly high heritability in this study – often not met under stress conditions (Ziyomo and Bernardo 2013) - makes direct selection under cold stress conditions in climate chambers worthwhile, considering an appropriate experimental setup and well-allocated resources. Similar results for beneficial direct selection have been reported both for upland rice (Venuprasad et al. 2007, Kumar et al. 2014) and tropical maize (Edmeades et al. 1999) under drought stress. On the other hand, in another study recurrent selection for cold tolerance in the field did not lead to a significant improvement of early seedling vigour in maize populations (McConnel and Gardner 1979) although the absence of appropriate low spring temperatures was recognised in that study. In fact there is no guarantee that on-field trials are hit by a cold weather period every year at the right time to allow direct selection for cold tolerance in the seedling or flowering stage. Therefore, a direct selection under artificial stress conditions appears to be beneficial when breeding for cold tolerant crops. Speaking from a practical point of view: if a genotype's seed batch exceeds the amount needed for field trials next season, screening tests for cold tolerance can be performed easily in climate chambers indoors in the off-season (additional Figure 1). Cold treatments can be precisely applied at stages of the plant's highest vulnerability if those are known. Given that cold tolerance is most needed for the early stages of soybean development until pod onset, experiments can be terminated after about two months in climate chambers. This way, also exotic soybean material from gene banks can be screened for cold tolerance (and other) traits in order to evaluate their aptitude as potential partners for novel crosses.

Genomic prediction might also prove beneficial in combination with experiments under artificial conditions. Naturally the available space in climatised greenhouses, phytotrones or climate chambers is scarce. Genomic prediction can be used to predict complex traits for individuals of a population by phenotyping a reduced set of selected genotypes in this limited space. Our study showed that for a complex trait such as cold tolerance, reduced test set sizes also yielded lower prediction accuracies but acceptable mean prediction accuracies were reached nonetheless if the set was reduced from 103 to 80 individuals. We also showed that prediction accuracy increased by including major trait QTL as fixed effects - especially for small test set sizes comprising 20 and 40 individuals. This observation is in line with Bernardo (2014), Boeven et al. (2016) and Spindel et al. (2016). However, one needs to be aware that confirmed QTL are required for the germplasm in question. Genomic prediction for yield and yield components was shown to be most effective within full-sib families and less so between individuals of unrelated families of triticale (Würschum et al.

2017). Thus, for practical breeding it appears possible to use a small number of random individuals from biparental populations as subsets for (cold) stress phenotyping in the off-season. Using genomic data, all individuals of the populations can be predicted. The most promising individuals from each population can be chosen for the next on-sesaon for the also limited and expensive field trial space. This reduction due to preselection would either allow for a decrease in work load in the field or an increase in the number of biparental populations that can be evaluated under field conditions. It would in turn improve the breeding process not just for soybeans in a practical sense.

No speed limit? - Requirements for speed breeding soybeans and the impact of light quality regimes on short-day crops

The proposed speed breeding protocol for LD crops by Watson et al. (2018) was adapted to the SD crop soybean in the following way: a day/night cycle of 10 h/14 h (28°C/28°C) and a light spectrum with increased blue light intensity, additional white and near-red light sources (<650 nm) of lower intensity and deprivation of far-red light enabled a short plant height and maturation of 8 soybean cultivars from different maturity groups within 77 days. These criteria can only be provided by climate chambers or greenhouses with a light control system - artificially and indoors.

Figure 5 from left to right: LED climate chambers for experimental purposes, soybean seedlings ~ 1 weeks after sowing in LED chamber, soybean plants ~ 3 weeks after sowing, soybean terminal flower ~ 4 weeks after sowing, soybean immature pods ~ 7 weeks after sowing. Photographed by W. L. Leiser 2019

Based on the results of our tests, the use and combination of spectrum-specific LED lamps is worthwhile. This requires specialised providers for lighting systems that are able to offer customised products based on the light demand of the crop. For example we discovered that the use of far-red light had negative effects on soybean's plant height, causing internode and petiole elongation and consequently increased the risk of lodging. This discovery is in line with the study of Hitz et al. (2019). In addition, we determined that plants for indoor speed

breeding should be kept as short as possible due to spatial limitations and the likelihood of multi-storey setups in practice. We concluded that far-red light should be excluded from a speed breeding light protocol that works with soybeans. On the other hand, we discovered a diametric effect of far-red light on the flowering behaviour for other SD crops: average flowering time for two sets of rice and amaranth cultivars was significantly shortened under the influence of far-red light. No such effect was observed in soybeans which is not in line with the proposals of Smith and Whitelam (1997), Childs et al. (1997) and Craig and Runkle (2012). It underlines the necessity to adjust the light protocol to the targeted crop. A ready-for-all speed breeding protocol for SD crops probably does not exist and requires crop-specific tests concerning day length and light quality parameters. Looking at the cultivars within the rice or amaranth sets, we noted phenotypic variation in flowering time: some genotypes were not significantly affected by the treatment while others were. We aimed to find a light protocol that would enable a quick but also a synchronised completion of life cycles for all cultivars included in our diversity panels. We found synchronisation a necessary criterion for logistical reasons: in order to make the light protocol available for future larger-sized speed breeding approaches, it would require a more or less equal maturity time for a broad spectrum of cultivars. Life cycle synchronisation of the genotypes was one of the main reasons why a SD protocol was used for soybeans because day lengths greater than 12 h significantly delayed the flowering times of some soybean genotypes. We were able to establish a soybean speed breeding protocol under controlled LED and climate conditions to meet the stated requirements. Building on this knowledge presented here, it may prove worthwhile to keep researching additional factors in order to exploit the full potential of this technique (for instance fertilisation or day-night temperature shifts) or further optimise the already investigated parameters (e.g. a more detailed investigation of the blue-(near)red ratio or growth stage dependent light). Due to the lower number of experiments conducted with rice and amaranth, the stated speed breeding conditions for these crops require even more investigation and research in the future in order to be optimised.

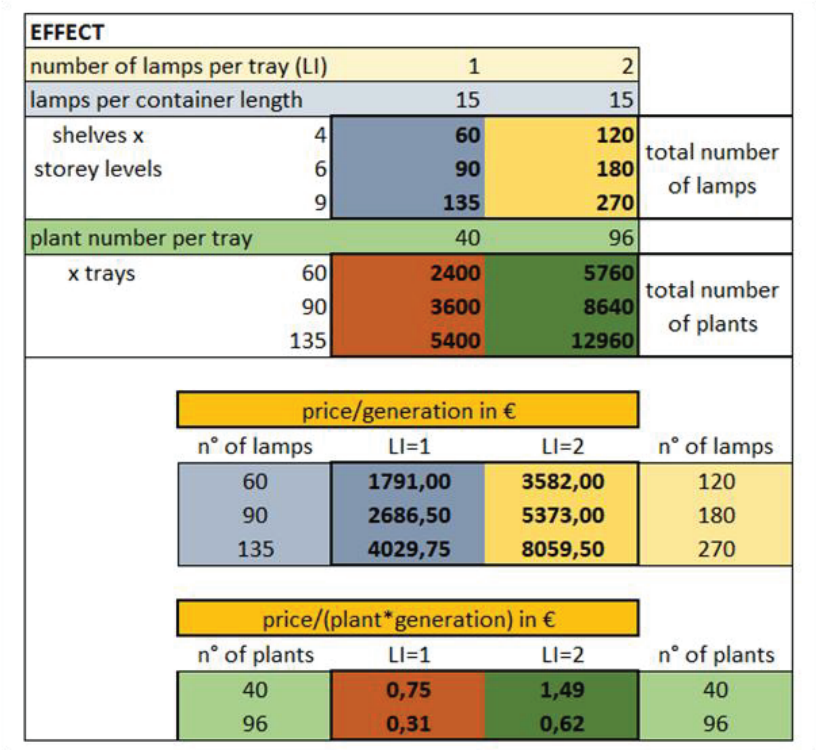
Economic and practical challenges of indoor speed breeding

For breeding companies it would be essential to know the monetary framework for an operating speed breeding system. Other tools that also reduce the breeding cycle like winter nurseries or doubled haploid line development, include cost intensive elements as well (e.g. shipment costs, station costs, labour costs, chemicals and laboratory equipment). But unlike speed breeding the economic dimensions of these tools are known and calculable due to years or decades of experience. Additional table 1 and figure 6 give an estimated overview of the lighting costs per genotype per generation cycle if a shipping container (11,6 m x 2,3

m x 2,5 m) is turned into a climate chamber. As fixed variables we assumed the power costs per kWh in Germany, the costs per lamp, their life expectancy, size and their wattage all based on the manual of one of the project's cooperating companies (https://www.growking.de/), the length of the day and length of the generation cycle both defined by the protocol for soybeans in this study. The costs per LED-lamp per breeding generation were calculated as 29.85 €, which is the combined price for purchase and operating costs per generation based on the lamp's life expectancy and operating hours per generation cycle (see additional table 1). Unknown to us, hence not included in the calculation are costs for temperature maintenance, soils and substrate, watering, fertiliser and costs for labour input.

We calculated prices for plant number per tray (40 or 96), the light intensity (one lamp or two lamps per tray), two or three rows of shelves within the chamber and two or three levels per shelf (figure 6). The energy and lamp purchase costs per plant per generation ranged from 0.31 € for the high plant throughput (96 plants per tray) and low light intensity scenario to 1.49 € per plant per generation for the scenario with lower plant throughput (40 plants per tray) and higher light intensity. In our experiments we could show that higher light intensities lead to increased germination rates when seeds from 63 days old plants were concerned, but this increase was not significant. Germination rates of the same level were achieved also with a lower light intensity and ~ 10 days older plants. Hence, the gained speed from a higher light intensity needs to be weighed carefully against the energetic costs of additional running days with less lamps.

These calculated energy costs from figure 6 should be compared to the costs for two-way shipment and station maintenance but without the labour costs when an off-season winter nursery is used for breeding purposes. Total costs (including labour costs) for one generation of soybeans grown in a tropical winter nursery in 2019/2020 were 0.73 € per plant (Hahn 2020) which at first glance appears to be financially more competitive to our calculated climate chamber scenarios. But even if the calculated energy costs of climate-control chambers exceed the expenses of a (sub)tropical winter nursery, other advantages that cannot be directly measured financially need consideration: There is the risk of unfortunate weather (such as a tropical storm) that has the potential to eradicate years of breeding effort while a controlled artificial system can operate more independently from the weather. The plant material remains on the station or close by, eradicating the risk of shipping delays to and from the tropical winter nursery which in turn reduces delays in the time frame of the whole breeding program. Plant tissue samples from genotypes grown in climate chambers can easily be obtained and genomic or marker analyses can run in parallel.



EFFECT				
number of lamps per tray (LI)		1	2	
lamps per container length		15	15	
shelves x storey levels	4	60	120	total number of lamps
	6	90	180	
	9	135	270	
plant number per tray		40	96	
x trays	60	2400	5760	total number of plants
	90	3600	8640	
	135	5400	12960	

price/generation in €			
n° of lamps	LI=1	LI=2	n° of lamps
60	1791,00	3582,00	120
90	2686,50	5373,00	180
135	4029,75	8059,50	270

price/(plant*generation) in €			
n° of plants	LI=1	LI=2	n° of plants
40	0,75	1,49	40
96	0,31	0,62	96

Figure 6 Overview of the calculated costs per plant per generation depending on the light intensity (LI=number of lamps per tray), number of shelves and storey levels and number of plants per tray.

Since the climate chamber is more or less hermetically sealed from the field, disease tests can be performed inside the chambers, the pathogen is kept off the field and chambers can be cleaned thoroughly after the experiments. Especially qualitative disease resistances, which are highly heritable, seem appropriate for testing, even in an early generation of a speed breeding protocol within a climate chamber. Screening resistant and susceptible lines in the off-season in a climate-control chamber seems advantageous for other reasons as well: Adjustment of favourable conditions for pathogen infestation – if they are known - seems relatively straightforward due to the controllable climate conditions. Infestation and scoring - work that is usually done in summer when other labour-intensive routines are to be done on-field – is relocated into the off-season. And finally and foremost, the screens would render valuable information to select for resistant lines or chose potential parental genotypes for future crosses. In combination with genomic data obtained from the plant material in the climate chamber, a QTL analysis can be done for example, which in turn could provide results that aid future MAS for those qualitative disease resistances. However, the introduction of pathogen screenings necessarily requires initial tests whether the speed breeding system, with its short and sturdy plants that are possibly already stressed in their small pots, allows an additional stressor to be introduced.

There are further challenges for speed breeding that have to be addressed. The costs for temperature maintenance remain a big question mark and although the efficiency of LED-lamps is superior compared to other lamps regarding light conversion from electric energy, cooling efforts are still required and will be higher the more LED-lamps are running simultaneously. As stated in the last chapter, our proposed protocol works under SD conditions. The risk of selecting photoperiod sensitive genotypes has to be considered here. It must however also be noted that in a standard single seed descent (SSD) breeding approach, no selection is performed in the early generations. And sooner or later also speed-bred lines are transferred from the climate chamber to field conditions. In that first year of field testing after speed breeding, the evaluation and selection of a high number of rather homozygous breeding lines requires a higher labour input given the missing selection. However, the breeding cycle in total will be shortened by speed breeding which may financially compensate for increased labour input.

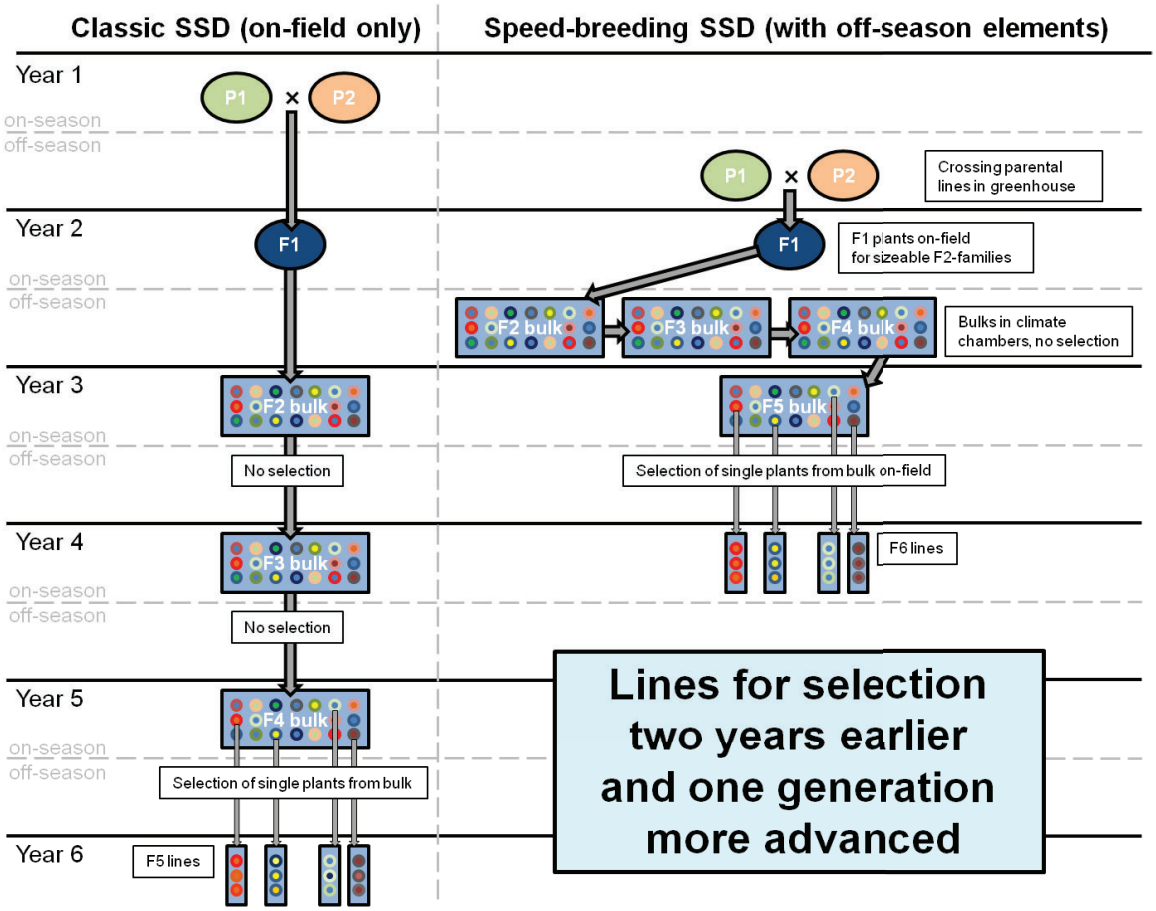


Figure 7 Timetable for breeding program following a standard SSD breeding program with on-field elements only (left) and a speed breeding SSD approach including off-season elements (indoor and controlled environment)

The biggest challenge remains when to integrate the speed breeding system into the breeding program. As stated earlier a standard SSD breeding protocol requires no selection

from F2 to F4 generation and it is here where speed breeding seems to fit the profile. For example: New soybean crosses can be performed in greenhouses in winter. The F1 generation from these crosses can then grow in the field from May to September to generate sizable F2-families. Afterwards, generation F2 to F4 are speed-bred in the off-season from October to mid-April (~230 days). This is possible given a ~ 75 day long generation cycle. The F5 generation is then grown and selected on field as usual. Thereby the breeding cycle from F1 to F4 was shortened from 3 years to 1 year (figure 7).

After the F5 generation there may be another possibility to use speed breeding conditions: Directly after the harvest of on-season field trials, a large part of the seeds from each plot is stored as usual during the off-season while a small part of each plot's seed batch can go into the climate chambers in order to perform two or three stress trials after one another (i.e. tests for cold tolerance, drought, flood or salt resistance, disease infestation). Results from these tests can be incorporated into the selection process. Simultaneously leaf samples for DNA extraction can easily be taken during one of those trials. Homozygosity in generation F6 or F7 is already at an advanced stage in self-fertilising crops (theoretically > 96 %). Genomic information from these soybeans grown during the off-season in the climate chamber may be a good proxy for the remaining seeds that were stored. The DNA obtained can be used for MAS or sequencing purposes. When the stored seeds grow in the next on-season, the collected phenotypic data can be combined with the obtained genomic data to conduct QTL analyses or aid GS purposes for example (additional figure 1).

Many laypersons and a handful of experts - Can citizen science support agricultural research?

The citizen science approach '1000 Gärten' with its 2492 locations was an unprecedented multi-location experiment regarding trial sites, logistics and the size of the data set. By including almost two and a half thousand private gardeners that were distributed equally amongst five latitudinal zones, valuable information concerning photoperiod requirement for soybeans in Germany was collected (figure 8 A). And by combining the gardener's data with publically available weather data, additional results for soybeans' temperature demands were obtained:

Most of the soybean lines flowered between mid-June and mid-July, the around two weeks before and after the summer solstice. Day length at that time of the year varies almost 1.5 h from the northernmost to the southernmost point in Germany. Hence, remnant photoperiodic sensitivity in the very early soybean material might have been visible when comparing the flowering dates between the five latitudinal zones. However no such flowering gradient from south to north was observed in the material (figure 8 A). The flowering rather followed a

temperature gradient in Germany with observed early flowering in the warmest areas of 2016 - along the Rhine valley between Mannheim and Mainz as well as in northern areas around Berlin - and the latest flowering with gardens positioned in generally colder (micro-) climates - close to the Alps, the Black Forest and the Swabian Jura (figure 8 B).

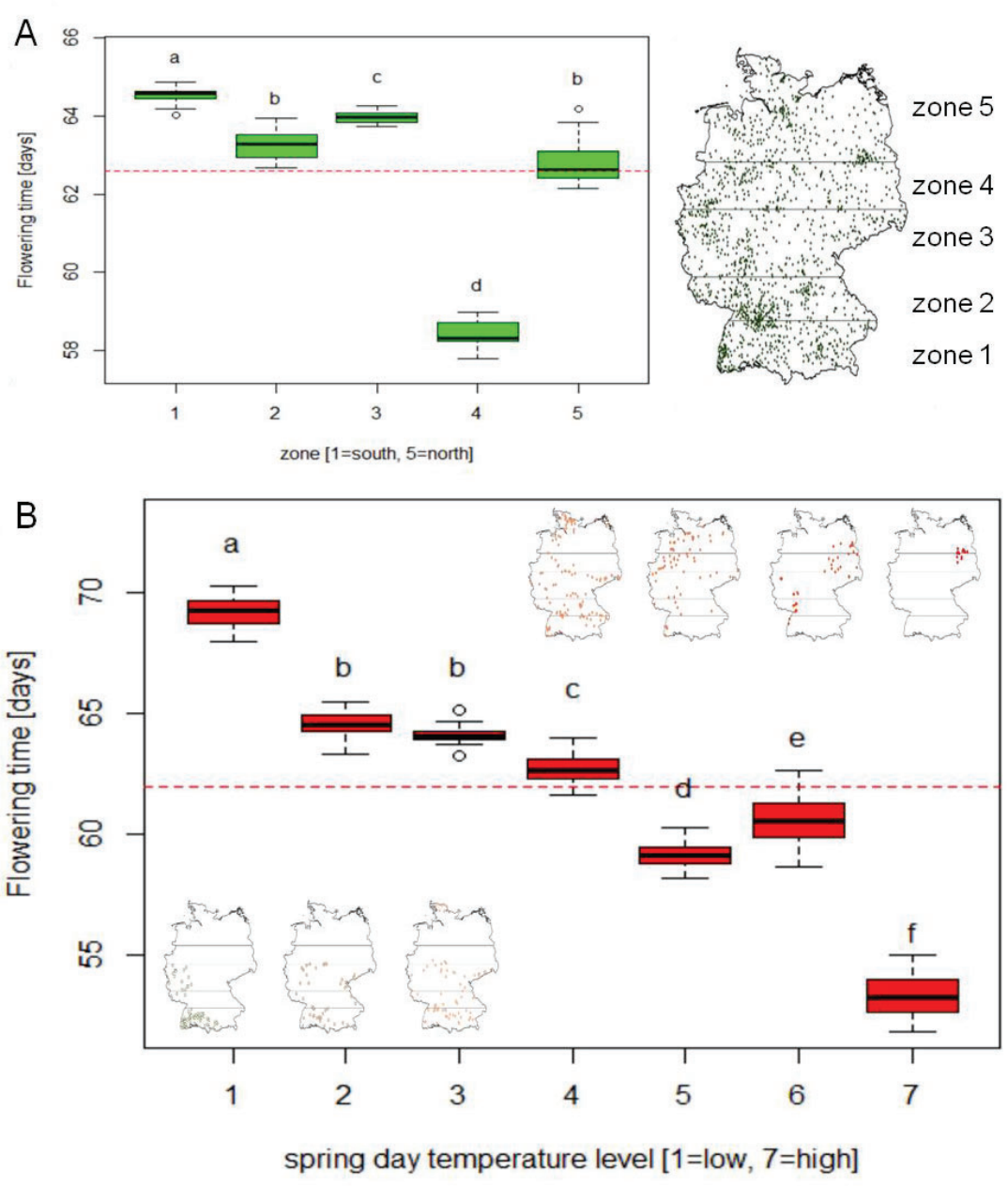


Figure 8 A: Boxplots showing flowering time of soybeans growing in the same latitudinal zone (left) and map of Germany with the latitude borders and positions of participating gardens (right). B: Boxplots showing flowering time of soybeans growing in the same temperature zone, maps of Germany above or below the boxes indicate the gardens positioned in the corresponding temperature zone, different letters on top of the boxes indicate significant differences of means according to Tukey's HSD test (α=0.05), red dashed line shows overall mean.

An almost identical pattern was observed for soybean maturity, indicating that within the very early maturity group segment (MG00 and MG000) not any more photoperiod insensitivity but rather temperature adaptation might be the trait to focus on when aiming for CE adapted cultivars in a breeding program. A deduction of that statement from a regular agricultural study involving only two or three field trial sites would have been more challenging if not impossible. This is one example which shows that hundreds of people can help answering specific agricultural questions about soybean growth in Germany simply by collecting data. Undoubtedly, their involvement requires additional workload to coordinate a project of such a scale: i.e. setting up the logistics and the database, explaining the purpose of the experiment so that participants can relate to the topic and find motivation to hold on, training the participants in the skills they need for collecting data. But when all that is carried out, a sizable and interesting dataset can arise.

There are challenges that need to be mentioned: A reduction of participant number throughout the course of the project was observed, which is in accordance with Worthington et al. (2012). This should be considered in the experimental design, the planning of the study and the analytic models because otherwise it will lead to missing data points or reduced replications. Participants need specific instructions and timely reminders on how and when to collect the data. Distribution of visual aids like pictures or videos, transmission of audio guides, invitations to blogs, forums or chat groups and regular reminder emails have to be considered in a citizen science setup. For plant breeding purposes there is also the practically important question when to incorporate the citizen science tool into a breeding program and two possibilities appear plausible:

- rather in the beginning of a breeding program with a still high number of lines distributed to many helpers to screen for highly heritable or qualitative traits (with the risk of losing information from the participants for some of the lines or having trouble to generate enough seeds for distribution at such an early stage of the program)
- or with a reduced number of lines to check for example yield stability across a high number of environments (which may require more space in the gardens, rigorous compliance with experimental setups and higher accuracy scoring the traits when more quantitative features are targeted by the citizens)

Surely a citizen science approach has its limits, particularly when highly specialised equipment or knowledge is required to answer a scientific question. Though the potential of citizen science is not yet fully tapped. Its usefulness as a supporting research tool should be considered. As of 2019 and only within Germany, 96 citizen science projects registered, connected and funded by the German Ministry of Education and Research are under way in

fields like nature and environment, history and tradition, education, society and health and medicine (Miyashita-Ostermann et al. 2019). And even in plant breeding ‘1000 Gärten’ as a citizen science project did not remain a ‘unicorn’. In 2018 a follow-up project ‘1000 Gärten 2.0’ for soybean breeding launched involving 1200 gardeners (http://www.1000gaerten.de/startseite/) in order to further advance adaptation of soybeans to CE climate conditions. In 2019 Sativa Rheinau started a citizen science project for lettuce breeding also with 1200 participants in Switzerland, France, Germany, Austria and the Benelux and have continued the project in 2020 as well (https://www.mit-vereinten-gaerten.org/). And also in 2019 van Etten et al. published a study that addressed crop variety changes during climate adaptation in Ethiopia, Nicaragua and India with in total 12409 crop plots supervised by farmers on their fields over a time period of two to five years. While these projects carry on, they become examples and consequently evidence for public funds and private capital that citizen science is a promising approach with future potential.

Summary

“With less than 2 years until the end of 2020, only 22% of EU+ soy use is responsible [...] and only 13% can be considered deforestation free [...]. At this stage of market development, soy is largely not traceable to origin.” (European Soy Monitor, 2017, Preface)

Although these numbers have increased by 16 and 6 %, respectively, according to the latest European Soy Monitor 2018 (European Soy Monitor, 2018), there seems to be a wide discrepancy in the EU between market demands and general sustainability aims regarding soybean products. Europe finally needs to sweep in front of its own front door as well, if it wants to maintain its protein demands and at the same time requests a reduction in the destruction of globally important tropical and subtropical ecosystems. One step towards more sustainable soybean products lies in the increase of domestic production which has the potential to decrease soybean imports from areas of unsustainable cultivation. An augmented EU production of soybeans can be achieved for example by increasing the yield potential of soybeans in areas where successful cultivation already takes place or by expanding the cultivation area to more northern parts of Central Europe. Breeding for new, improved and adapted soybean cultivars that meet those terms, is a key activity towards that aim. This dissertation elucidates three different ways how the adaptation of soybeans to the climatic and photoperiodic conditions of Central Europe can be assisted and even accelerated:

1) By using off-season climate-controlled LED chambers to enable a speed breeding single seed descent approach. A 10 h light regime, rich in blue and deprived of far-red light emission is capable to significantly reduce and synchronise the generation time of soybeans. It was possible to shorten the life cycle for a panel of 8 soybean cultivars from different maturity groups to 77 days. This allows several generations of soybeans to be grown within one year. For the short day crops rice and amaranth on the other hand, different light quality parameters were favoured. In those crops mean flowering time was accelerated when far-red light was included in the light protocol. This underlines the importance of a crop-specific light regime in order to realise the full potential of LED-based speed breeding single seed descent.

2) By including experiments in climate-control chambers in combination with molecular tools (i.e. genomic prediction) to advance cold tolerance in soybeans. This quantitatively inherited key trait is necessary to adapt soybeans to colder regions and consequently extend growing areas of this crop to higher latitudes in Europe. In the biparental soybean population Merlin × Sigalia (103 recombinant inbred lines) three QTL for cold tolerance during pod onset were found on chromosomes 7, 11 and 13. The relatively small proportion of genotypic variance

for this trait explained by these QTL underlines the quantitative nature of cold tolerance. Genomic prediction was shown to be a promising approach to select for cold stress tolerance. Scenarios with different test set sizes and prediction models were evaluated. In scenarios with smaller test set sizes prediction accuracies increased if known and confirmed QTL were included in the prediction model.

3) By incorporating citizen science into the breeding process. The citizen science project ‘1000 Gärten’ from 2016 approached this topic. Phenotypic data from soybean cultivars and breeding lines were collected by citizen scientists in 2492 gardens throughout Germany which generated a unique dataset. Among many other results this study was able to show that in 2016 and within the early maturity segment of soybeans the factor temperature influenced flowering and maturity to a higher degree than photoperiod although day length differed by over an hour between the north and the south of Germany during the time of flowering. It was shown that this admittedly challenging tool can realise a significant impact not only regarding the possibility of a highly multi-environmental screening of breeding material but also by connecting plant breeding, agriculture and potential future costumers in order to raise awareness and acceptance of a crop in larger parts of the society - a factor that may not be highlighted enough when a new crop is introduced to our agriculture.

These approaches should not be seen as an alternative to classical plant breeding, but rather considered as valuable additional tools that can contribute to conventional breeding of soybeans, as well as other crops. If applied, the presented tools may assist plant breeding to pave Europe’s way towards a greener and more sustainable future that is urgently needed.

Zusammenfassung

„In weniger als zwei Jahren bis Ende 2020 können nur 22% der EU + -Sojaverwendung als nachhaltig [...] und nur 13% als abholzungsfrei angesehen werden [...]. In dieser Phase der Marktentwicklung ist die Herkunft von Sojabohnen weitgehend nicht zu verfolgen.“ (European Soy Monitor, 2017, Preface)

Obwohl diese Zahlen laut aktuellem European Soy Monitor 2018 (European Soy Monitor, 2018) um 16 bzw. 6% gestiegen sind, scheint es in der EU bei Sojaprodukten eine große Diskrepanz zwischen den Marktanforderungen und den sich selbst gesetzten allgemeinen Nachhaltigkeitszielen zu geben. Europa muss endlich auch vor seiner eigenen Haustür kehren, wenn es seinen Proteinbedarf aufrechterhalten will und gleichzeitig die Zerstörung global wichtiger tropischer und subtropischer Ökosysteme anprangert. Ein Schritt in Richtung nachhaltigerer Sojaprodukte liegt in der Steigerung der innereuropäischen Produktion. Das hat das Potenzial die Sojaimporte aus nicht nachhaltigen Produktionsgebieten zu verringern. Eine erhöhte Sojabohnenproduktion in der EU kann erreicht werden, indem beispielsweise das Ertragspotenzial von Sojabohnen in Gebieten erhöht wird, in denen bereits ein erfolgreicher Anbau stattfindet, oder indem Anbaugebiete auf nördlichere Teile Zentraleuropas ausgedehnt werden. Die Züchtung neuer, verbesserter und angepasster Sojasorten, die diese Bedingungen erfüllen, ist ein Schlüsselelement, dieses Ziel zu erreichen. In dieser Dissertation werden drei verschiedene Möglichkeiten aufgeführt, welche die Adaptation von Sojabohnen an die klimatischen und photoperiodischen Bedingungen Zentraleuropas unterstützen und sogar beschleunigen können:

1) Durch die Verwendung einer klimatisierten „off-season“ LED-Kammer, um einen „speed breeding“ Ansatz für Einzelsamenramsch zu ermöglichen. Ein 10-stündiges Lichtregime, welches reich an blauem Licht ist und kaum Emissionen im fernroten Spektralbereich aufweist, kann die Generationszeit von Sojapflanzen erheblich verkürzen und synchronisieren. Es war möglich, den Lebenszyklus von acht ausgewählten Sojabohnensorten unterschiedlicher Reifegruppen auf 77 Tage zu reduzieren. Dadurch können mehrere Generationen Sojabohnen innerhalb eines Jahres wachsen. Die Kurztagpflanzen Reis und Amaranth bevorzugten jedoch andere Lichtqualitäten. Bei diesen Kulturarten wurde die mittlere Zeit bis zur Blüte beschleunigt, wenn fernrotes Licht in das Lichtprotokoll integriert wurde. Dieser Fund hebt die Bedeutung kulturartspezifischer Lichtregime hervor, um das volle Leistungsvermögen eines LED-basierten „speed breeding“ Ansatzes zu nutzen.

2) Durch Einbeziehung von Experimenten in Klimakammern in Kombination mit molekularen Werkzeugen (z.B. genomic prediction), um die Kältetoleranz von Soja zu verbessern. Dieses quantitativ vererbte Schlüsselmerkmal ist notwendig, um Sojabohnen an kältere Gebiete anzupassen und folglich das Wachstum dieser Kultur in nördlichen Breiten Europas zu fördern. In der biparentalen Sojabohnenpopulation Merlin × Sigalia (103 rekombinante Inzuchtlinien) konnten drei QTL auf den Chromosomen 7, 11 und 13 für Kältetoleranz zur Zeit des Hülsenansatzes gefunden werden. Der relativ kleine Anteil der erklärten genotypischen Varianz dieser drei QTL an dem Merkmal unterstreicht die genetisch quantitative Beschaffenheit von Kältetoleranz. Genomic prediction wurde als erfolgsversprechender Ansatz eingeschätzt, um auf Kältestresstoleranz zu selektieren. Szenarien mit unterschiedlich großen Testsets und Vorhersagemodellen wurden bewertet. Die Vorhersagegenauigkeit konnte in den Szenarien mit kleineren Testsetgrößen erhöht werden, indem bekannte und bestätigte QTL dem Vorhersagemodell hinzugefügt werden konnten.

3) durch die Einbeziehung von Bürgerwissenschaft (Citizen Science) in den Züchtungsprozess. Das 2016 gestartete Bürgerwissenschaftsprojekt „1000 Gärten“ nahm sich dieses Themas an. Dabei wurden phänotypische Daten von Sojasorten und Sojazuchtlinien von Bürgerwissenschaftlern in 2492 Gärten aus ganz Deutschland erfasst und gesammelt, welche zu einem einzigartigen Datensatz zusammenflossen. Es konnte gezeigt werden, dass dieses zugegebenermaßen herausfordernde Werkzeug hinsichtlich der Möglichkeit eines Screenings von Zuchtmaterial an vielen Standorten einen signifikanten Einfluss erzielen kann. Unter anderem konnte in dieser Studie gezeigt werden, dass im Jahr 2016 und innerhalb des frühen Reifegruppesegments bei Sojabohnen der Faktor Temperatur ausschlaggebender auf das Blüh- und Reifeverhalten war als die Photoperiode. Und das obwohl die Tageslänge zwischen Nord- und Süddeutschland zur Zeit der Blüte mehr als eine Stunde Unterschied aufweist. Zusätzlich konnte durch die Verbindung von Pflanzenzüchtung, Landwirtschaft und potenziellen zukünftigen Kunden das Bewusstsein und die Akzeptanz für Soja als Kulturart in einem größeren Teil der Gesellschaft erhöht werden - ein Umstand, welcher möglicherweise nicht genügend Beachtung findet, wenn eine neuartige Kulturart in unsere Landwirtschaft eingeführt wird.

Diese Ansätze sollten nicht als Alternative zur klassischen Pflanzenzüchtung verstanden werden, sondern als wertvolle zusätzliche Instrumente, die zur konventionellen Züchtung von Sojabohnen, aber auch anderer Kulturen beitragen können. Im Bereich der Pflanzenzüchtung kann die Anwendung ebendieser hier vorgestellten Instrumente dazu beitragen, Europas Weg in eine grünere und nachhaltigere Zukunft zu ebnen, die dringend benötigt wird.

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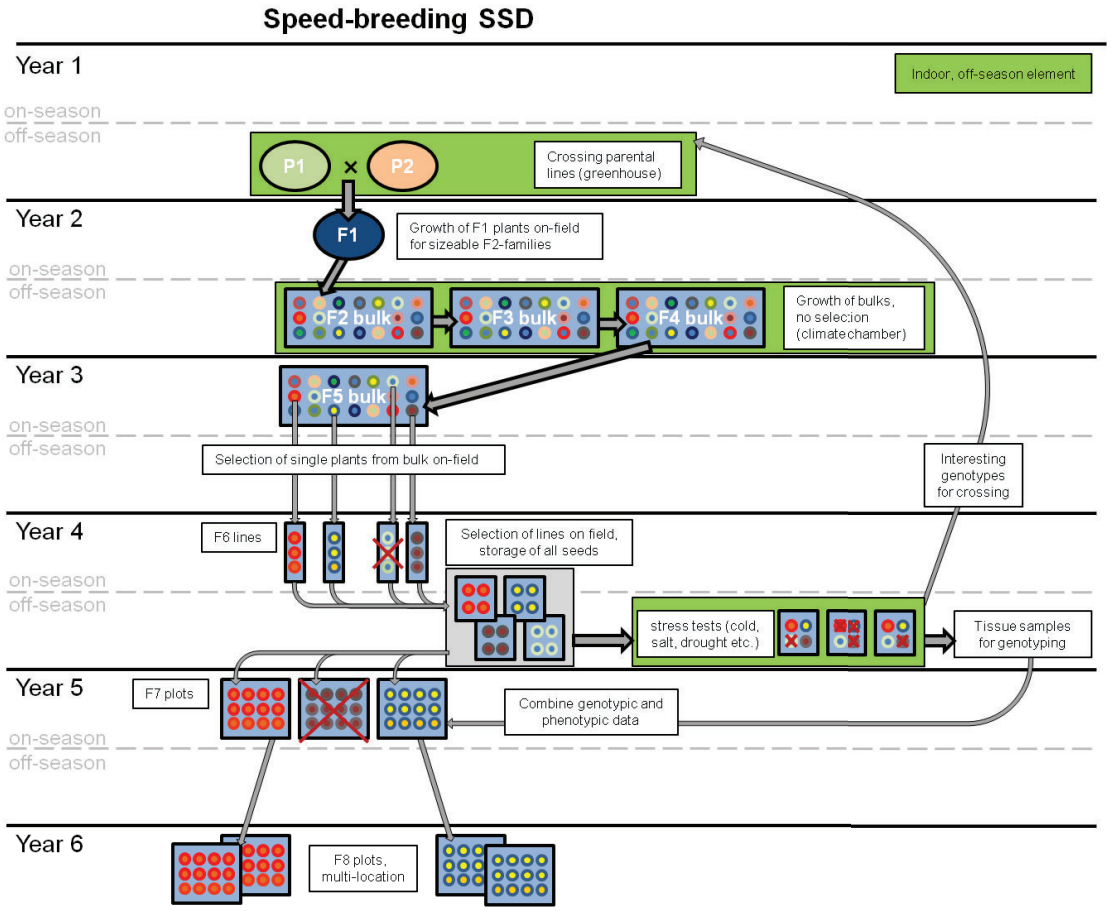
Appendix

Additional table 1: Overview of fixed, varying and calculated factors that were used for an estimate calculation of the power costs (figure 6) of a shipping container that is turned into an off-season, climate-controlled LED chamber.

FIXED		
container		
length	11,6	m
width	2,3	m
height	2,5	m
lamps		
purchase	190	€
length	0,75	m
life	50000	h
wattage	120	W
protocol		
daylength	10	h
cycle length	75	d
power		
costs	0,3	€/kWh

VARYING			
light intensity	1	2	lamps/tray
shelves	2	3	number
storey levels	2	3	number
plants	40	96	number/tray

CALCULATED		
costs per lamp		
purchase costs per hour	0,0038	€/h
purchase costs per gen.	2,85	€/generation
wattage per day	1200	W*h
wattage per generation	90000	W*h/generation
kWh per generation	90	kWh/generation
kWh costs per gen.	27	€/generation
costs per lamp per gen.	29,85	€/generation



Additional figure 1 Timetable and extended flow chart for a possible breeding scheme following a speed breeding SSD program with on-field and off-season elements (indoor and controlled environment).

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