Plant Soil (2022) 475:237–252
https://doi.org/10.1007/s11104-022-05364-x

REVIEW ARTICLE

# A quantitative review of soybean responses to waterlogging: agronomical, morpho-physiological and anatomical traits of tolerance

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Received: 16 September 2021 / Accepted: 26 February 2022 / Published online: 24 March 2022

**Abstract** The impact of waterlogging on crops has increased during the last decades due to climate change, being considered the second most important abiotic stress after drought in soybean, with yield reductions of up to 80%. In this review, we performed a quantitative review from 54 papers about waterlogging effects in soybean. Yield losses are greater (57%) with waterlogging occurring at reproductive stages between R1 and R3 compared to vegetative stages, which is mostly related to a diminished number of pods per plant. Waterlogging impacts on biological nitrogen fixation, with losses of about 30 and 50% in nodule number and weight, with the greatest reductions in reproductive stages. The nitrogenase activity is drastically reduced with water excess but can quickly restore after. Losses in photosynthesis are about 30–50%, mostly related to reduced stomatal conductance and poor N status. Soybean can also develop aerenchymatous tissue in roots, hypocotyls and nodules, which may alleviate the anoxia generated by waterlogging by facilitating oxygen transport. Further efforts should be invested in investigating the effect of waterlogging on nodulation and the impact and subsequent recovery of root growth.

**Keywords** Soybean · Waterlogging · Yield · Dry weight · Aerenchyma · Adventitious roots · Nodulation

## Introduction

Climate change has impacted food, fiber, and energy production over the last decades (Asseng et al. 2009), increasing the frequency and intensity of extreme events, including large rainfall events (Hirabayashi et al. 2013). In this scenario, waterlogging due to excessive rainfalls and poor soil drainage results in a significant threat for agriculture in many parts of the world, affecting a total of *ca.* 1700 million hectares (Konnerup et al. 2018; Voesenek and Sasidharan 2013). Waterlogging is defined as prolonged soil saturation with water at least 20% higher than the field capacity (Aggarwal et al. 2006). This soil saturation determines a low-oxygen atmosphere (i.e., hypoxia) that limits the yield of many crops in humid areas, especially when high rainfall is associated with

Responsible Editor: Al Imran Malik.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11104-022-05364-x.

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flat topography (Collaku and Harrison 2002), high water tables and poor soil drainage (Jitsuyama 2017). Waterlogging impacts around 10–12% of agricultural soils (Kaur et al. 2020), and about 6 million tons of grain per year are lost due to this stress with economic losses of approximately $1.5 billion annually (Wu et al. 2020).

Soybean is a major source of protein and oil for humans and livestock; and it is also used in the industry for biodiesel, biocomposites for construction, clean and beauty products (Singh 2010). It also represents more than half of the vegetable oil and two-thirds of it as protein meal consumed worldwide, resulting in the most important legume crop (Divito et al. 2015). With a total production of approximately of 350 million tons worldwide and a harvested area of *ca.* 130 million hectares (FAO 2019), soybean is one of the grain crops with highest production in the world, concentrated in Brasil (33%), USA (28%) and Argentina (16%) with *ca.* 76% of the world production (FAO 2019). Waterlogging ranks second (after drought) in abiotic stresses causing significant losses in different crops, including soybean production (Valliyodan et al. 2017). The estimated average yield losses in soybean range from 40 to 80%, depending on the phenology (Phukan et al. 2016).

In this review, we quantitatively review the responses of soybean to waterlogging in terms of (i) yield and dry weight accumulation, (ii) biological nitrogen fixation (nodulation and nitrogenase activity) and (iii) plant physiology (carbon fixation, water relations, antioxidants, aerenchyma and adventitious rooting). We focus on differences in the abovementioned variables regarding the impact of waterlogging occurring in different moments of the crop cycle, stress duration, and tolerance variability among genotypes. To do this, we created a database of 54 articles (published in SCI-indexed journals) that evaluated the effect of waterlogging on soybean. Of those studies, 44% assessed yield responses to waterlogging, 41% evaluated shoot dry mass as affected by water excess, while only 24% examined the impact of hypoxia on root dry mass. In 50% of these reports, waterlogging was imposed using pots with a substrate, 4% were done using nutrient solutions, and 46% were carried out under field conditions. Waterlogging depths ranged from water at the soil surface (25% of experiments) to 20 cm above the soil surface, involving different degrees of partial submergence of plants (75% of experiments; studies performing complete shoot submergence were not included for this contribution).

Data used to calculate the waterlogging-induced reductions on the analyzed variables were extracted directly from Tables (when available) or Figures (see Supplementary Table S1, S2, S3 and S4). In the case of Figures, values were extracted using the software GetData Graph Digitizer v2.26 (http://getdata-graph-digitizer.com/). Non-parametric tests were used as data were not normally distributed. So, to describe the distribution of the data, the medians and the first and third quartiles were used. The mean was also included for comparison in some cases. Wilcoxon Signed Rank Test (non-parametric test) was used to compare each case's median against a hypothetical median. In all cases, the inclusion of the variables in the analyses depended on the availability of enough data to satisfy the requirements of the tests (e.g., it is not advisable to run the Wilcoxon Signed Rank Test when 'n' is below five).

## Dry weight responses

### Yield and its numerical components

The effect of waterlogging on grain production is strongly dependent on the phenological stage at which it occurs (Figs. 1 and 2). The least impact produced by the stress is observed at early vegetative stages (V1-V4), with a reduction that ranges between 25 and 40% (median = 29%) of controls (Fig. 2). Variations in the duration of waterlogging during early vegetative stages also lead to a differential levels of yield loss; as plants waterlogged at V4 for four days showed losses of 10.5% on average while waterlogging for 7 and 14 days led to reductions of 15.5 and 41.5% of controls, respectively (Scott et al. 1989). Waterlogging in V5-V8 stages shows a wider range in yield reduction, including moderate reductions as in V1-V4 stages, but also with higher reductions, between 25 and 60% (median = 34%; Fig. 2); which is consistent with being transitional stages between vegetative and reproductive phases. In line with what occurs in the earlier stages, waterlogging duration also leads to variations in yield reductions; plants waterlogged at V5 showed higher reductions when the treatment lasted four days, compared to two days of waterlogging (reductions of 36% vs. 28%; Pedó

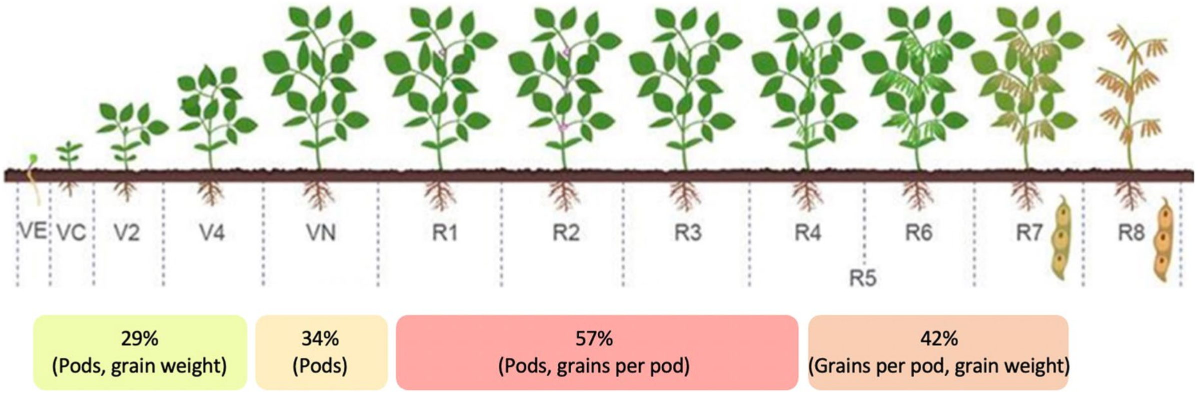

**Fig. 1** Reductions in yield (as % of control) and, in parenthesis, the main numerical components affected by waterlogging in different stages of the soybean cycle (Fehr and Caviness 1977)

et al. 2015). Yield losses are the highest, with waterlogging occurring at early reproductive stages (from flowering, R1, to pod setting, R3), with reductions of 55–60% (median = 57%; Fig. 2). Independently of waterlogging duration, plants showed 49% and 54% reductions after four and eight days of treatment at R2, respectively (Rhine et al. 2010). Lastly, with water excess at late reproductive stages (R4-R7), the yield is less reduced by waterlogging than in the previous stages and shows a wider range between 35 and 50% (median = 41.5%; Fig. 2). This is probably associated with the fact that, at these late stages, the number of pods and grains are almost established and that the grain weight is generally less changing under stress than the former variables. Waterlogging duration differentially affects the yield also, with shorter durations (up to 13 days) lowering yield by 37–47% of controls on average, and longer durations (more than 14 days) decreasing yield to 80–83% of controls (Table S5).

The numerical components (number of pods per plant/ha, number of grains per pod and grain weight) differentially influence yield under waterlogging (Fig. 3). The component that mainly influences yield is the number of pods, positively correlating with grain production ($r^2 = 0.73$; Fig. 3a). The effect of waterlogging on the number of pods is also affected by the genotype. For instance, two contrasting parentals showed reductions in pods per plant by 19–25% and 72–85% of controls after 21 days of waterlogging at V3, while the average of the 156 RILs of these genotypes showed losses of 49–52% (Githiri et al. 2006). Specifically, the influence of the genotype is also evident with waterlogging occurring at later vegetative stages, with larger differences in pod reductions among genotypes when waterlogged from V5 to V9 (reductions of 9, 15 and 41% in 'Enrei', 'Sakukei 4' and 'En1282') compared to waterlogging occurring in more sensitive stages, from V6 to R1 (reductions of 57, 61 and 77% in 'Enrei', 'Sakukei 4' and 'En1282'; Matsunami et al. 2007). The number of grains per pod does not correlate with yield ($r^2 = 0.004$, Fig. 3b), as this numerical component seems to be quite conservative. As an example, soybean subjected to water deficit showed reductions in the number of seeds like the ones on the number of pods, so the ratio remained the same as controls (Momen et al. 1979). The reduction in grain weight is reflected in a significant reduction in yield, (see $r^2 = 0.60$, Fig. 3c), which suggests that the latter, together with the number of pods explain most of the yield losses after waterlogging. Reproductive stages are far more sensitive to waterlogging, with higher reductions in this trait; as an example, seven days of waterlogging lowered grain weight by 8–15% when it occurred at V2, V3 or V7. In contrast, up to 62% reductions were observed with the same waterlogging duration at reproductive stages, being R3 the most sensitive one (Linkemer et al. 1998).

### Shoot and root dry weights

Overall, the effects of waterlogging on the shoot and root dry weights are similar, with reductions ranging from 30 to 35% (median = 30%) and 30 to 40%

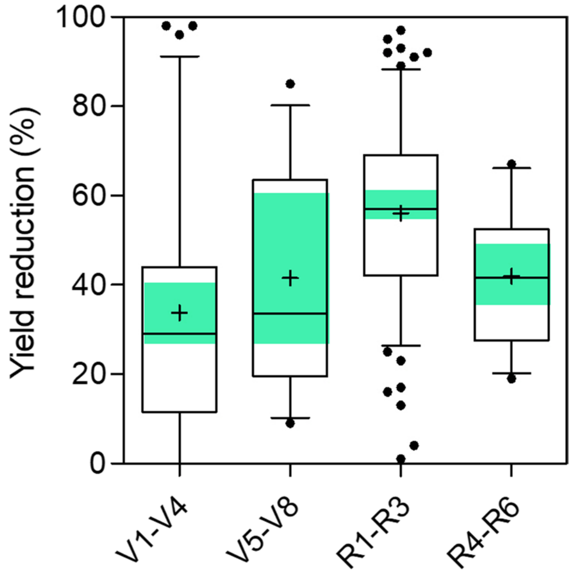

**Fig. 2** Reductions in yield (as % of controls) due to waterlogging occurring during vegetative stages [V1-V4 ($n=37$) or V5-V8 ($n=24$)) and reproductive stages (R1-R3($n=76$) or R4-R6($n=12$)] in soybean (*Glycine max* (L.) Merr.). Boxes are 50% of the observations with the median shown as the horizontal line within each box, mean as '+' within each box, and bars extending from each box are 10 and 90 percentiles; outliers are shown as ●. Data are from plants in pots/containers ($n=8$) and field conditions ($n=16$) reported in 23 peer-reviewed articles (data values, key experimental conditions, and references are given in Supplementary Table S1). Waterlogging (days) for experiments with the various species were (ranges with median in parentheses) 2–23 (7). Waterlogging depths ranged from the soil/substrate surface to 20 cm above the surface and in all cases without complete shoot submergence. The green shaded area in each bar denotes reductions in yield in different phenological stages for different ranges as a percentage of controls based on Wilcoxon signed-rank tests where: V1-V4: 25% < median < 40%; V5-V8: 25% < median < 60%; R1-R3: 55% < median < 60%; and R4-R6: 35% < median < 50%

(median = 35%), respectively (Fig. 4a). Nevertheless, the negative impact can differ depending on the moment of waterlogging occurrence. To illustrate, waterlogging for ten days at late vegetative stages (V6 or V8) drastically reduced the shoot dry weight by 65% and 60–79% (respectively); while waterlogging at V3 reduced it far less (by 23–60% of controls), with no reductions when plants were waterlogged at V1 (Matsunami et al. 2007). Something similar occurred with the root dry weight, with reductions of up to 26% and 35% of controls with waterlogging at V1 or V3, and losses of 38–75% and 53–75% at V6 or V8, respectively (Matsunami et al. 2007). The lower reductions in growth with early vegetative waterlogging might be related to the longer recovery periods. Additionally, losses in shoot and root dry weight also vary according to the genotype, showing variability in tolerance to waterlogging. For example, Buttery (1987) found that waterlogging for 29 days at 30 DAS (vegetative stage) in sensitive genotypes led to significant losses in shoot and root dry weights, with reductions of 77–78% and 63–85%; while a tolerant genotype showed higher tolerance with 19% and 38% lower shoot and root dry weights, respectively.

Reductions in root dry weight are closely correlated to those in shoot dry weight ($r^2$: 0.51). The shoot to root ratio, as a rough estimator of the potential balance between transpiration and water uptake, depends on the dry weight reductions (% of controls; Fig. 4b). The waterlogging duration differently affects dry weight responses. With waterlogging at V6, seven days of treatment led to losses in shoot dry weight of 14%, while 21 days provoked higher losses of 26% of controls (Bacanamwo and Purcell 1999a). However, with longer waterlogging durations than 14 days at V2, the reductions on dry weight do not seem to differ (comparing a 14-day with a 28-day-waterlogging), with losses of 45 and 66% in shoot and root dry weights, respectively (Henshaw et al. 2007a, b).

In summary, the yield is drastically reduced (median of 57%) with waterlogging occurring between R1 and R3 when pod and grain number are established; while when waterlogging occurs from R4 to R6, yield decreases about 42% on average due to reductions in grain per pod and grain weight. Early vegetative stages (V1 from V4) are less affected by waterlogging, with reductions of 29% mainly due to lower pod number and grain weight. Lastly, the stress occurring between V5 and V8 leads to intermediate decreases of 34% related to losses in pod number. Shoot and root dry weight reductions generally range between 30 to 40% of controls. Still, responses also vary according to the timing of waterlogging, with greater losses observed in late vegetative and reproductive stages compared to early vegetative ones. Regarding duration of waterlogging, there is evidence that longer stresses derive in higher dry mass reductions. As example, Scott et al. (1989) observed that plants waterlogged at V4 for four days reduced the shoot

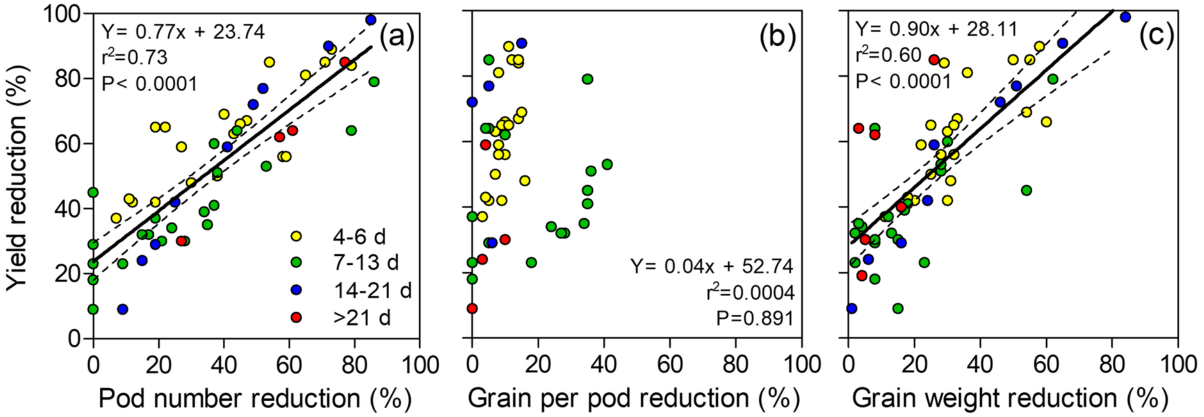

**Fig. 3** Relationship between yield reductions (as % of controls) and pod number reduction (a, $n=54$), grain number per pod reduction (b, $n=46$) or grain weight reduction (c, $n=55$) due to waterlogging in soybean (*Glycine max* (L.) Merr.). The fitted relationship in (**a**) and (**c**) is a linear of the type y = ax+b. Yellow, green, blue, and red dots indicate 4–6, 7–13, 14–21 and more than 21 days of waterlogging duration. Values shown were taken from Supplementary Table S1

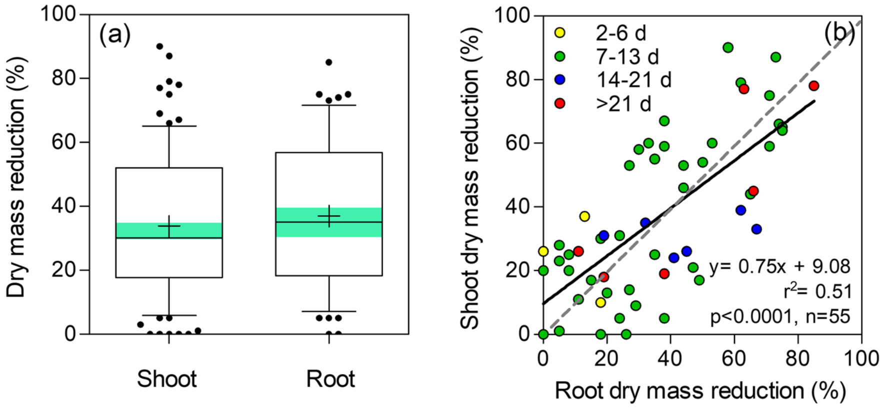

**Fig. 4** Effect of waterlogging on shoot ($n=97$) and root ($n=55$) dry mass reductions (as % of controls) (a) and the relationship between shoot and root dry mass reductions (b, $n=55$). Boxes are 50% of the observations with the median shown as the horizontal line within each box, mean as '+' within each box, and bars extending from each box are 10 and 90 percentiles; outliers are shown as ●. Data are from plants in pots ($n=12$), hydroponic culture ($n=1$) and field conditions ($n=9$) reported in 22 peer-reviewed articles (data values, key experimental conditions, and references are given in Supplementary Table S2). Waterlogging (days) for experiments with the various species were (ranges with median in parentheses) 3–29 (10). Waterlogging depths ranged from the soil/substrate surface to 20 cm above the surface and in all cases without complete shoot submergence. The green shaded area in each bar in (**a**) denotes reductions in dry mass (as % of controls) for different ranges based on Wilcoxon signed-rank tests where: shoot, 30% < median < 35%; and root, 30% < median < 40%; The fitted relationship in (**b**) is a linear of the type y = ax+b. The grey dotted line indicates the 1:1 relationship between both variables. Yellow, green, blue, and red dots indicate 2–6, 7–13, 14–21 and more than 21 days of waterlogging duration

dry mass by 10–29%, while waterlogging occurring for two weeks lead to losses of 34–52%. The same happened with waterlogging at R2, with reductions of 9–30% after four days of waterlogging, contrasting with 21–65% reductions after two weeks of stress (Scott et al. 1989).

## Biological nitrogen fixation (BNF) responses

### Nodule number, weight and nitrogenase activity

Waterlogging can produce several damages to BNF, even as harmful as water deficit stress (Santachiara et al. 2019). Sallam and Scott (1987) reported that flooding during the vegetative stages (i.e., V1 stage) completely inhibited soybean nodulation, as at least a minimal oxygen availability (0.0016 atm) is required for optimal nitrogen fixation (Keister and Rao 1977). Moreover, the lack of oxygen suppresses nitrogenase activity in soybean roots (Sprent 1969; Minchin and Pate 1975). The number and total weight of the nodules are similarly affected by waterlogging, with reductions ranging from 30–50% (median = 43%) and 35–50% (median = 39%), respectively (Fig. 5a, b). The negative effect of waterlogging on nodule dry weight (NDW) depends on its duration as it is almost doubled (from 38 to 71% reductions of controls) with a more extensive period of stress (14 vs 28 days at V2; Henshaw et al. 2007a). In this regard, four days of waterlogging at R5 reduced the NDW by 27–59%, while ten days at R1 led to 59–68% losses in this trait (Sung 1993). The opposite happens with the nodule number (NN), with more significant reductions of 41–72% after four days of waterlogging at R5, compared to 27–29% with ten days of treatment at R1 (Sung 1993), probably related to the fact that, independently of the water regime, R1 is a stage with higher nodulation development than later reproductive stages, like R4 (Lamptey et al. 2014). Regarding the moment of occurrence, waterlogging at vegetative stages (i.e. V1) for seven days reduced NDW by 42%, while plants waterlogged at reproductive stages (i.e. R4) suffered from losses of 74% of controls. Similarly, the NN was highly reduced with waterlogging at R4 (reductions of 63%), while at V1, this trait was maintained the same as controls (Yamane and Iijima 2016). In addition, waterlogging occurring for ten days at V1 led to losses of up to 33% in NDW, while the stress at V8 implied reductions of up to 81% of controls (Matsunami et al. 2007). Also, waterlogging at V1 reached lower reductions in NN (of up to 52%) compared to occurring at V8 (losses of up to 82%, Jung et al. 2008). Apart from restraining nitrogen supply to plants, with a lowered number of nodules the carbon sink strength is also reduced, which could lead to negative feedback in the photosynthetic rate, as it happens in other legume with BNF, faba bean, subjected to different water regimes (Parvin et al. 2020).

The nitrogenase activity (NA) shows a slightly wider range of reductions than NN and NDW, with values from 30 to 55% lower than controls (Fig. 5c). With waterlogging, $O_2$ levels are drastically depleted, and since this molecule is a terminal acceptor of electrons in the mitochondria, its scarcity is related to NADH accumulation and ultimately with a suppressed ATP production (da-Silva and do Amarante

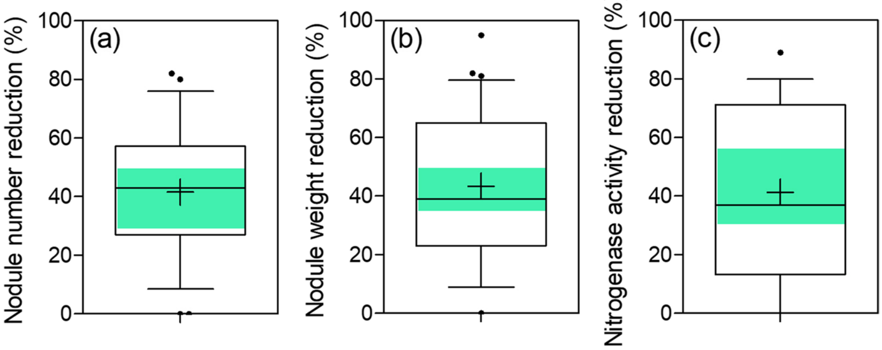

**Fig. 5** Impact of waterlogging on reductions in nodule number (as % of controls) (a, $n = 24$), nodule weight (b, $n = 31$) and nitrogenase activity (c, $n = 18$) in soybean (*Glycine max* (L.) Merr.). Boxes are 50% of the observations with the median shown as the horizontal line within each box, mean as '+' within each box, and bars extending from each box are 10 and 90 percentiles; outliers are shown as ●. Data are from plants in pots ($n = 9$) and field conditions ($n = 2$) reported in 11 peer-reviewed articles (data values, key experimental conditions, and references are given in Supplementary Table S3). Waterlogging (days) for experiments with the various species were (ranges with median in parentheses) 3–29(10). Waterlogging depths ranged from the soil/substrate surface to 5 cm above the surface and in all cases without complete shoot submergence. The green shaded area in each bar denotes nodule number and weight, and nitrogenase activity reductions (as % of controls) for different ranges based on Wilcoxon signed-rank tests where a, 30% < median < 50%; b, 35% < median < 50%; and c, 30% < median < 55%

2020). Nitrogenase is an enzyme extremely sensitive to oxygen, so a gas diffusion barrier is delimited in the nodule cortex to carefully regulate the amount of oxygen inside the nodules together with the leghemoglobin synthesis (a protein that helps with oxygen diffusion to bacteroid); making BNF an energetically highly expensive process, with an elevated respiratory rate (Vance and Heichel 1991; Roberts et al. 2010; Olivares et al. 2013). Thus, it is expected for the nitrogenase activity to be negatively impacted by reductions in ATP production after waterlogging. Related to the latter, with waterlogging at 36 DAS for five days, the NA was wholly inhibited (no activity was detected); but later, it was recovered up to controls a few days after waterlogging ended (Maekawa et al. 2011). In this respect, plants with 21 DAS waterlogged for ten days suffered from losses of 89% in NA (Bacanamwo and Purcell 1999a). The moment of waterlogging occurrence also seems to determine the degree of damage in NA, with mild reductions when the stress for ten days occurred at V1 (0–8%), and higher losses at V3 and V6 (25–63% and 64–75%, respectively; Jung et al. 2008). Differences also exist among genotypes with diverse tolerance to waterlogging. Sung (1993) observed that a tolerant genotype waterlogged for ten days at R1 showed reductions of only 27%, which were much lower than in the sensitive one (79% of controls); but without differences when waterlogged for four days at R5 (reductions of 36 and 38% of controls).

To summarize, the effect of waterlogging on nodule number and weight is similar, with losses ranging between 30 and 50% of controls, with more significant reductions after higher stress durations and with waterlogging occurring in reproductive stages. The nitrogenase activity is a very sensitive variable to anoxia, being reduced almost to zero but with a high recovery capacity during post-waterlogging.

## Physiological responses

### Net photosynthesis and associated variables

Photosynthesis is reduced by 30–55% (median = 41%) due to waterlogging (Fig. 6a). Garcia et al. (2020) found this variable to be very sensitive to a 7-day-waterlogging at V4 in the five analyzed genotypes, with reductions ranging from 34 to 50%, and full recovery after a one-week-recovery. Similarly, plants waterlogged at V4 for nine days showed 63–73% reductions in both genotypes used. Still, the authors found differences in tolerance between cultivars after waterlogging at R2, with a tolerant genotype reducing photosynthesis by 38%, and a sensitive one with losses of 73% of controls (Cho and Yamakawa 2006). Yamane and Iijima (2016) observed differences in photosynthesis reductions due to waterlogging occurring in V1 and R4, with greater impact after the 7-day-waterlogging applied at R4 (losses of 75%) than at V1 (reductions of 32%). Reductions in stomatal

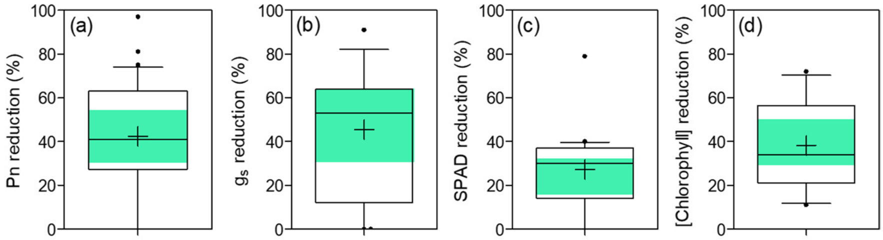

**Fig. 6** Impact of waterlogging on reductions in net photosynthesis (Pn) (as % of controls) (a, $n = 34$), stomatal conductance ($g_s$, b, $n = 19$), leaf greenness (SPAD, c, $n = 23$) and chlorophyll concentration (d, $n = 17$) in soybean (*Glycine max* (L.) Merr.). Boxes are 50% of the observations with the median shown as the horizontal line within each box, mean as '+' within each box, and bars extending from each box are 10 and 90 percentiles; outliers are shown as ●. Data are from plants in pots ($n = 14$) and field conditions ($n = 3$) reported in 17 peer-reviewed articles (data values, key experimental conditions, and references are given in Supplementary Table S4). Waterlogging (days) for experiments with the various species were (ranges with median in parentheses) 3–21(9). Waterlogging depths ranged from the soil/substrate surface to 20 cm above the surface and in all cases without complete shoot submergence. The green shaded area in each bar denotes Pn, $g_s$, SPAD and [chlorophyll] reductions (as % of controls) for different ranges based on Wilcoxon signed-rank tests where a, 30% < median < 55%; b, 30% < median < 65%; c, 15% < median < 35%; and d, 30% < median < 50%

conductance, a variable that strongly regulates photosynthesis in $C_3$ species, show a higher variation, with reductions ranging from 30 to 65% (median = 53%) of controls (Fig. 6b). Oosterhuis et al. (1990) observed almost the same range of reductions in both photosynthesis and stomatal conductance after seven days of waterlogging at V4 (losses of 52–58%) or R2 (losses of 57–63% of controls), suggesting that the degree of stomata closing could be strongly limiting carbon fixation. In line, plants waterlogged for eight days at vegetative stage showed very similar reductions in photosynthesis and stomatal conductance, being 81 and 82%, respectively (Pereira et al. 2020); and the same occurred in plants waterlogged for ten days at R3, with 97 and 91% losses for these variables (de Marcos Lapaz et al. 2020). Waterlogging duration also influences stomatal conductance, with three days of treatment leading to losses of 33% of controls, and with five days of waterlogging increasing reductions to 80% of controls (Maekawa et al. 2011).

Nitrogen (N) status also conditions carbon fixation in soybean (Boote et al. 1978); with leaf greenness (SPAD) and chlorophyll concentration as indicators of the latter. SPAD presents reductions by waterlogging that range between 15 to 35% (median = 30%) of controls (Fig. 6c). Chlorophyll concentration drops more drastically than SPAD after waterlogging, showing reductions of 30 to 50% of controls (Fig. 6d). Consistently with the fact that carbon fixation is affected by restrictions in $CO_2$ supply in the first place (Farquhar and Sharkey 1982); changes in SPAD and chlorophyll concentration are less pronounced than stomatal conductance. As an example, waterlogging at R3 for ten days led to losses of 97% in photosynthesis, with reductions of 51% in chlorophyll concentration (de Marcos Lapaz et al. 2020); also, after eight days of waterlogging at 30 DAS, plants showed losses of 81% and 34% in photosynthesis and chlorophyll concentration, respectively (Pereira et al. 2020). Similarly, losses of 75% in photosynthesis coincided with 40% reductions in SPAD after seven days of waterlogging at R4 (Yamane and Iijima 2016). Additionally, chlorophyll concentration shows variability among genotypes; for instance, three cultivars with differential tolerance reduced this variable by 21, 12 and 35% after three days of waterlogging at V5 (Calvin et al. 2019). SPAD also shows variability in the responses to waterlogging among different genotypes, with three tolerant cultivars that maintained SPAD like controls and three sensitive ones with 37–39% reductions after a 14-day-waterlogging at V4 (Kim et al. 2019).

## Water status responses

The effect of waterlogging on variables related to water status such as leaf water potential and transpiration rate has been scarcely addressed in soybean. Garcia et al. (2020) found that plants waterlogged at V4 for seven days showed variability in their leaf water potential responses depending on the genotype; two tolerant genotypes showed a 10–38% raise than controls at the end of waterlogging, restoring values to controls at seven days post-waterlogging. Although showing higher values than controls in water potential, these genotypes showed reductions of 19–35% in the transpiration rate after seven days of waterlogging, with this variable restored seven days after. Contrastingly, the other three (sensitive) genotypes showed 2–42% lower water potential (i.e., restored during recovery only in one genotype), with reductions of 26–45% in the transpiration rate at the end of the treatment, recovered in all genotypes. Another study also showed an increase in leaf water potential after seven days of waterlogging at 70 DAS (30% higher than controls), but with reductions of 45% in the transpiration rate (Araki 2006). The higher leaf water potential in waterlogged plants regarding controls could be explained by the early stomatal closure triggered by abscisic acid aiming to prevent excessive water loss due to transpiration (Pantin et al. 2013). In addition, Oosterhuis et al. (1990) seemed to have worked with relatively tolerant genotypes, with ‘Essex’ waterlogged for seven days at V4 showing 15% higher water potential than controls, and ‘Forest’ with values similar regarding controls. When waterlogged at R2 for seven days, those genotypes showed 13–15% lower values than controls. Differences in tolerance to waterlogging between genotypes were also observed by Cho and Yamakawa (2006), where waterlogging at V4-V5 or R2 reduced the transpiration rate by 28–32% in the tolerant genotype, while in the sensitive the stress reduced this variable by 55–61% compared to controls.

### Antioxidant responses

Antioxidant enzymes, like catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) help in preventing the accumulation of reactive oxygen species (ROS). ROS, such as hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O^{\cdot}_2$) and $O_2^{\cdot -}$ radicals, provoke oxidative stress and can derive in fatty acid and protein oxidation, negatively affect nucleic acids, inhibit certain enzymes and lead to permanent metabolic disorder and programmed cell death (Inzé and Van Montagu 1995; Hossain et al. 2015; Andrade et al. 2018). The CAT (which detoxifies $H_2O_2$) activity from leaf samples was reduced by 37% of controls on average after one week of waterlogging at V4, with a peak of higher values than controls after a two-day- recovery, followed by partial recovery five days later (Garcia et al. 2020). When examined in root samples, the effect on CAT activity was even higher than in leaves because although reductions were like those on shoots, this trait could not be restored even seven days post-waterlogging (Garcia et al. 2020). Reductions in CAT activity in leaves were also reported by Yamane and Iijima (2016), where plants waterlogged for one week at V1 showed 38% lower activity. Still, no differences were found after waterlogging at R4. Some differences were found among genotypes in their response to waterlogging, with reductions of 84% in CAT of leaves in the most sensitive genotype, and losses of 21% of controls in the tolerant genotypes (Garcia et al. 2020).

Additionally, it was observed that a 7-day-waterlogging at V4 raised on average 80% the SOD (which detoxifies $O_2^{*-}$) activity in shoot samples, showing values like controls after seven days post-waterlogging (Garcia et al. 2020). On the contrary, waterlogging on root samples led to a SOD activity lowered by 17% on average but was fully restored during recovery in four of the five genotypes used (Garcia et al. 2020). Moreover, the decrease of SOD activity was also reported in cotyledonal tissue by Sidhu et al. (2020), in which 4-day-old seedlings subjected to 24 h of waterlogging showed 20% lower enzyme activity.

The activity of APX, which have a key role in $H_2O_2$ scavenging in the cytosol and chloroplasts (Inzé and Van Montagu 1995), was maintained at control values in leaf samples of plants after seven days of waterlogging at V4 and during recovery; while in root tissues this trait was lowered by 37% on average, with a successful recovery one week after (Garcia et al. 2020). Another study showed an 11% reduction in APX activity in leaves of plants waterlogged at V1 for one week and a similar decrease (8% of controls) with waterlogging at R4 (Yamane and Iijima 2016). Roots also showed greater sensibility to waterlogging, with APX activity lowered by 67% after a 3-day-treatment in 2-day-old-seedlings (Shi et al. 2008); in contrast with the APX activity in cotyledons of plants of similar age waterlogged for 24 h (Sidhu et al. 2020) that remained with similar values to controls.

A study by Kim et al. (2018) registered the effect of two days of waterlogging in soybean plants at V2 on the activity of glutathione [GSH, a metabolite which scavenges $OH^{\cdot}$ and $^1O_2$, (Gill et al. 2013)] and glutathione reductase [GR, an enzyme that helps to maintain a high level of GSH, (Gill et al. 2013)]. Waterlogged plants showed 81% lower GSH and similar GR levels than controls in root samples after two days of treatment, while in shoot samples, on the contrary, GSH was reduced by 17%, and GR activity decreased by 84% of controls (Kim et al. 2018).

In summary, waterlogging reduces photosynthesis in a range between 30 and 55%, along with similar decreases in stomatal conductance, with reductions being in general enhanced in reproductive stages compared to vegetative ones. N status is also impacted by waterlogging through losses in leaf greenness and chlorophyll concentration between 15–35% and 35–50%, respectively. However, plants can cope with a depleted physiological behavior by activating antioxidant enzymes like catalases, superoxide dismutases and ascorbate peroxidases, which help to eliminate reactive oxygen species (ROS).

## Adventitious roots and aerenchyma formation

Several studies reveal that plants can cope with waterlogging stress by developing aerenchyma and adventitious roots when waterlogged, increasing their porosity, and facilitating the diffusive transport of oxygen along roots under flooded conditions (Colmer 2003; Striker 2012). Aerenchyma formation is triggered by ethylene accumulation due to restrictions in gas diffusion in the waterlogged soils and followed by increased ROS levels in tissues, which contribute to the programmed cell death and cell wall degradation leading to the generation of aerenchyma lacunae (Yamauchi et al. 2013). Those set of responses finally generate a programmed cell death, leading to a rapid generation of lysigenous aerenchyma in the cortex of adventitious roots (Thomas et al. 2005; Yamauchi et al. 2013). So, from this perspective, the generation of ROS could lead to beneficial changes that help in coping with waterlogging. In fact, $H_2O_2$ due to its stability and high diffusion capacity through cell membranes, is known to have a key role in plants as a signaling molecule, conferring advantages related to stress acclimation and antioxidative defense (Hossain et al. 2015). In the case of nodulated legumes, as soybean, studies show beneficial effects of aerenchyma associated with $N_2$ fixation under waterlogging, related to a pathway to facilitate the gas diffusion to the submerged nodules, such as oxygen (and mitigating the losses in ATP production) and $N_2$ to be fixated and converted to nitrates (Walker et al. 1983; Loureiro et al. 1995; James and Crawford 1998; Shimamura et al. 2002; Thomas et al. 2005). Bacanamwo and Purcell (1999b) observed that roots from soybean plants waterlogged for 21 days at 28 days after sowing (DAS) produced adventitious roots representing 33.3% of the total root dry weight (controls did not show presence of adventitious roots). On average, these roots presented 14.9% of porosity due to aerenchyma formation, while controls showed only 1.1% of porosity. Additionally, soybean waterlogged for ten days at V6 showed a root porosity of 21.7% (vs 0.4% in controls), while the nodule porosity increased 2.5-fold compared to controls (Thomas et al. 2005).

Adventitious root production is dependent on the waterlogging duration; Henshaw et al. (2007a) observed that the adventitious root dry weight was equivalent to 2.5% of total plant weight (average of 10 RILs) after two weeks of waterlogging-induced hypoxia at V2, which was increased up to 4% after four weeks of waterlogging. Kim et al. (2015) also observed a higher adventitious root number after ten days of waterlogging, compared to those developed after five days of treatment; also combined with genotypic variability, as one genotype showed 17 vs 3 roots per plant, respectively; and the other developed 2 and 9 roots per plant after five and ten days, respectively. However, with long-term exposure to waterlogging aerenchyma developed similarly despite using different treatment durations; with two weeks of waterlogging at 10 DAS leading to similar results than with five weeks of treatment (63% and 68% of aerenchyma, respectively, which also enhanced the $O_2$ partial pressure in roots). The latter contrasted with what occurred to plants under drained conditions, which did not develop aerenchyma (Shimamura et al. 2010). So, this study also suggests that aerenchyma formation response saturates in the first 1–2 weeks of water excess.

In soybean, the enhanced oxygen diffusion under the hypoxic environment observed in many genotypes is mostly given by the ability of developing secondary aerenchyma (i.e., aerenchymatous phellem) in the hypocotyl, tap root, adventitious roots and nodules that is constitutively produced during prolonged waterlogging (Jackson and Armstrong 1999; Shimamura et al. 2003; Yamauchi et al. 2013). The secondary aerenchyma is related to the development of a spongy white tissue around roots, stems and nodules (Yamauchi et al. 2013). Plants can develop secondary aerenchyma a few weeks after waterlogging through phellogen development (secondary meristem), aerenchymatous phellem cell development and elongation of those cells (Yamauchi et al. 2013). Apart from maintaining the oxygen flow through the root system, another advantage of secondary aerenchyma in waterlogged environments is the ability to vent toxic substances from the soil, such as avoiding accumulation of $CO_2$ (Shimamura et al. 2010). As examples of phellem formation, early development of secondary aerenchyma was observed in plants waterlogged for 14 days at VC (cotyledon stage; Shimamura et al. 2003), with 30% porosity versus 10% registered in controls of the cv. 'Aso aogari'. Consistently, Takahashi et al. (2018) found that 10-day-old seedlings waterlogged for seven days could generate aerenchymatous phellem, which represented 2.4 times the stele area. Secondary aerenchyma development also depends on the waterlogging duration, with a developed tissue area of 8.4 $mm^2$ with two weeks of waterlogging (and 1.5 $mm^2$ in controls) vs 43.2 $mm^2$ after five weeks of treatment (Shimamura et al. 2010).

Among the traits that can ameliorate the effect of waterlogging, aerenchyma development facilitates oxygen transport through roots. Studies inform that soybean can generate aerenchyma in roots, and that this process can be enhanced with longer stress durations. Importantly, this crop can also develop 'phellem' (i.e., secondary aerenchyma) in stems, roots, and nodules after prolonged waterlogging, providing better tissue oxygenation and helping to vent toxic substances from the waterlogged soil.

## Conclusion and future outlook

Plant physiology, growth and yield in soybean are adversely affected by soil waterlogging. The magnitude of the hypoxia-induced negative effects depends on genotype, plant developmental stage and the environmental conditions (i.e., temperature and related atmospheric evaporative demand). The highest yield penalization due to waterlogging occurs at early reproductive stages (i.e., R1 to R3), while the water excess during early vegetative stages (i.e., V1 to V4) causes significantly less impact on yield. Important genotypic variation is evident in physiological responses to low oxygen, such as reductions in photosynthesis, stomatal conductance, leaf chlorophyll concentration, and activation of critical antioxidant enzymes (i.e., SOD, CAT, GR, APX). Genotypes also differ in the numbers of adventitious roots developed and the porosity (i.e., aerenchyma) and thus in the potential movement of oxygen into and along the roots. Nodule number and weight, and nitrogenase activity are reduced by waterlogging, but some variations exist among genotypes, although the reports in these regards are still scarce. N deficiency in leaves can explain the reduced growth during waterlogging, and it could slow plant recovery post-anoxia. The ability to recover upon drainage following waterlogging is important for yield. The time after the stress is also important, with lower yield reductions after a longer recovery period.

The Fig. 7 shows an integrative scheme that relates the variables affected by waterlogging in soybean analyzed along this study. Briefly, it can be said that environmental variables that change upon waterlogging, like oxygen depletion in soil and increased ethylene concentrations can trigger a series of plant morpho-physiological and anatomical adjustments, such as the synthesis of antioxidant enzymes that scavenge ROS, adventitious root formation and aerenchyma development, which regulate the oxygen flux within the roots. In turn, restrictive oxygen levels can drastically reduce the BNF (lowering nodule number and weight, and diminishing nitrogenase activity), since it is a high energy demandant process and ATP production is negatively affected by hypoxia; and water and nutrient absorption by roots can also be minimized because of the latter. Thus, physiological processes like photosynthesis and transpiration can be reduced due to stomatal closure in the first place, and later also because of chlorophyll degradation linked to an anticipated leaf senescence. Lastly, dry mass accumulation can be strongly reduced, leading to plants with lower root and shoot weights, which could directly implicate losses in yield (mainly due to reductions in the number of pods and grain weight).

We suggest three priority areas for research on traits that could contribute to breeding more waterlogging tolerant genotypes of soybean: (i) N plant status (chlorophyll retention, and efficiency of N uptake) after waterlogging, (ii) aeration of roots, hypocotyl, and nodules via primary and secondary (i.e., phellem) aerenchyma under flooded conditions and (iii) identification of critical traits defining the recovery ability following transient waterlogging. Also, progress in identifying QTL associated with waterlogging and seed submergence tolerance in soybean (*e.g.*, Ye et al. 2018; Nguyen et al. 2021) can unlock paths, with further work, for possible marker-assisted selection and detection of specific genes contributing to tolerance. In particular, the recent identification of a QTL associated with root system architecture and plasticity (*qWT_Gm03* in Ye et al. 2018) demonstrated improvement in waterlogging tolerance and yield. This work adds up to the ones that identified the markers linked to Rps genes or QTL conferring resistance to *Phytophthora sojae*, which is a pathogen favored by water excess in soils (see Nguyen et al. 2012). Previous works have already mapped 11 quantitative trait loci for root development (root length, root surface area, root diameter, and change in average root diameter, among others) under hypoxia conditions (Nguyen et al. 2017). In addition, the utilization of wild relatives (e.g., *Glycine canescens*, *G. clandestina*, *G.latifolia*, *G. microphylla*; see Mammadov et al. 2018) as shown in *Zea nicaraguensis* for maize (Abiko et al. 2012; Mano and Omori 2013; Pedersen et al. 2021) to introduce some traits (e.g., root constitutive aerenchyma), could improve waterlogging tolerance, and these wide crosses could also deliver some new resources for gene discovery. Lastly, much remains to be learned concerning nodulation and root growth recovery following waterlogging and

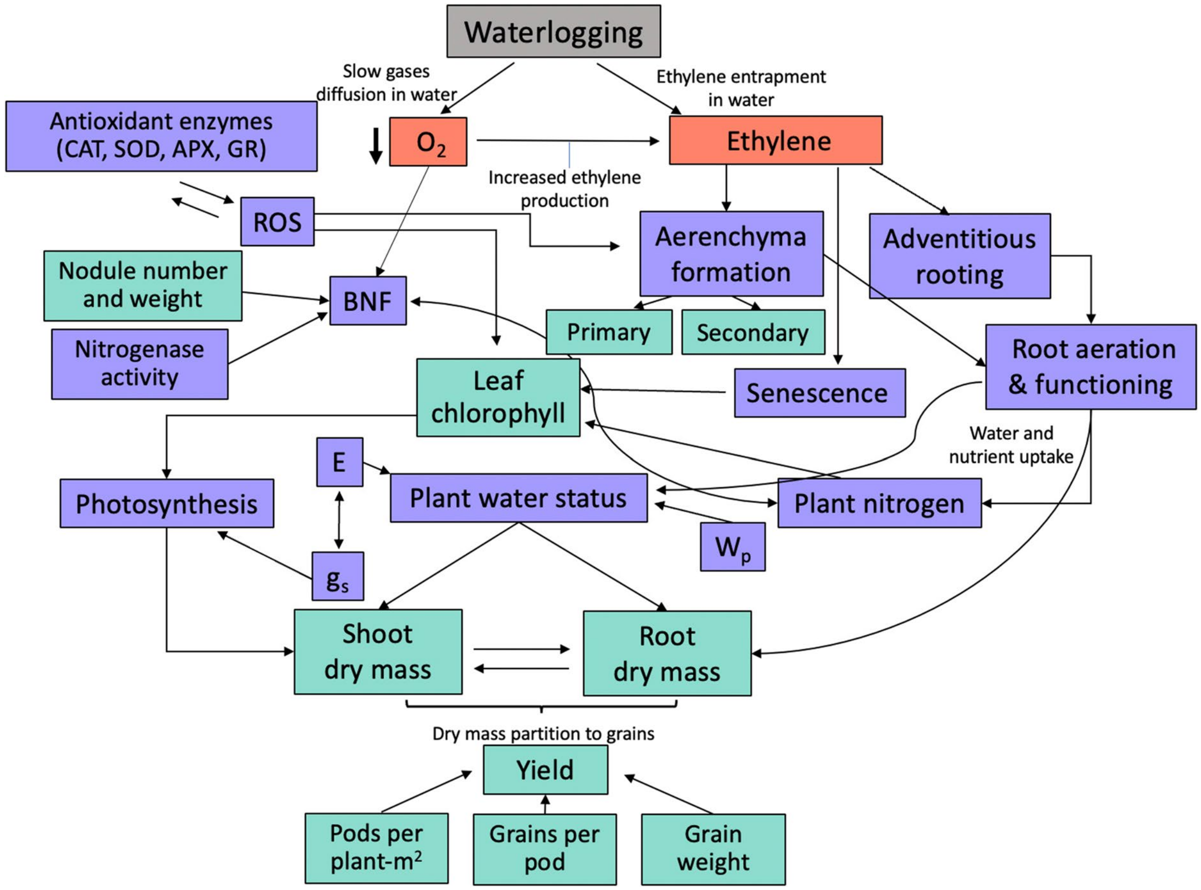

**Fig. 7** Scheme summarizing the effect of waterlogging on soybean. The orange boxes contain soil-related variables: ethylene and oxygen ($O_2$) soil concentrations. The violet boxes indicate flow variables, such as plant morpho-physiological and anatomical processes and variables related: enzyme activity (catalases-CAT-, superoxide dismutases-SOD-, ascorbate peroxidases-APX-, glutathione reductases-GR-), reactive oxygen species-ROS- activity, biological nitrogen fixation -BNF- (and nitrogenase activity), aerenchyma and adventitious root formation, root aeration and functioning, senescence, plant nitrogen and water status, water potential-$W_p$-, transpiration rate-E-, stomatal conductance-$g_s$- and photosynthesis. The green boxes represent stock variables: nodule number and weight, primary and secondary aerenchyma, leaf chlorophyll, shoot and root dry masses, yield, and its components (number of pods, grains per pod and grain weight)

whole-plant recovery more generally, both of which show some apparent genotypic variation.

In future studies, complementary approaches should be made at field, plant and molecular levels, so a comprehensive and clearer understanding of waterlogging responses in soybean can be reached. Although detailed experiments, focusing on specific metabolic processes, lack the realistic approach compared to field experiments given the controlled environment, they allow us to identify key plant features that can improve waterlogging tolerance. On the other hand, to fully test that those features are reproducible in a natural and productive environment, it is crucial to perform field experiments. Even though converting the knowledge produced in a laboratory, a growth chamber or a glasshouse (controlled conditions) to the field is not simple, strategies like imitating some resources of the field in controlled experiments like photothermal ratio, the type of soil used, or plant density could help to make the level transition better. Additionally, a tool like modelling the aboveground and subterranean environment could also be useful to make the transition between controlled experiments and the field easier (Poorter et al. 2016).

**Authors' contributions** RP made the database. RP, DM and GS wrote the manuscript.

**Funding** RP has a postdoctoral fellowship from CONICET (Argentina).

**Data availability** Not applicable.

**Code availability** Not applicable.

**Declarations**

**Ethics approval** Not applicable.

**Consent to participate** All the authors give their consent to participate.

**Consent for publication** All the authors give their consent for publication.

**Conflicts of interest** The authors have no conflict of interest.

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