

Rothamsted Repository Download

A - Papers appearing in refereed journals

Oddy, J., Raffan, S., Wilkinson, M. D., Elmore, J. S. and Halford, N. G. 2020. Stress, nutrients and genotype - understanding and managing asparagine accumulation in wheat grain. *CABI Agriculture and Bioscience*. 1, p. 10.

The publisher's version can be accessed at:

- <https://dx.doi.org/10.1186/s43170-020-00010-x>
- <https://rdcu.be/b5ULO>

The output can be accessed at: <https://repository.rothamsted.ac.uk/item/98139/stress-nutrients-and-genotype-understanding-and-managing-asparagine-accumulation-in-wheat-grain>.

© 28 July 2020, Please contact library@rothamsted.ac.uk for copyright queries.

REVIEW

Open Access



Stress, nutrients and genotype: understanding and managing asparagine accumulation in wheat grain

Joseph Oddy¹, Sarah Raffan¹, Mark D. Wilkinson¹, J. Stephen Elmore² and Nigel G. Halford^{1*} 

Abstract

Plant stress and poor crop management strategies compromise the foundations of food security: crop yield, nutritional quality and food safety. Accumulation of high concentrations of the amino acid asparagine in its free (soluble, non-protein) form is an example of an undesirable outcome of stress for the nutritional quality and food safety of wheat because of its role as a precursor to acrylamide, a carcinogenic processing contaminant. In this review, we cover what is known about the mechanisms and functions of free asparagine accumulation in the grain during normal development and particularly during stress in wheat. Comparisons with other plant species, yeast, and mammals are drawn in order to gain deeper insight into the conserved biology underlying asparagine accumulation. Crop management strategies and practices are discussed in the context of managing asparagine accumulation, which must be balanced against other desirable goals, such as sustainability, protein content and yield.

Keywords: Asparagine, Food security and nutrition, Wheat, Plant stress, Signalling, Function, Crop management, Nitrogen mobilisation, Ammonia detoxification

Background

The amino acid asparagine has long been of interest to plant biologists because of its role in nitrogen transport and stress responses (Lea et al. 2007). In the early 2000s, however, asparagine in crops gained new significance because of the discovery that free (soluble, non-protein) asparagine is a precursor for the food processing contaminant, acrylamide. Acrylamide forms from free asparagine and reducing sugars, mainly glucose, fructose and maltose, during a non-enzymatic reaction called the Maillard reaction. This reaction occurs when food is heated above 120 °C under low moisture content in processes such as frying, roasting, baking and toasting (Mottram et al. 2002; Stadler et al. 2002). In wheat products,

free asparagine concentration determines the potential for acrylamide formation (reviewed in Raffan and Halford (2019)).

Dietary acrylamide intake is concerning because of its links to cancer (reviewed in Raffan and Halford (2019)) and authorities such as the European Commission have been prompted to introduce regulations on acrylamide levels in food (European Commission 2017). Food manufacturers have adapted their processes and applied more effective quality control measures to reduce the levels of acrylamide in their products, but there is a limit to what can be achieved with that approach without affecting product quality. In order to make further improvements they need raw materials with consistently low potential for acrylamide formation. Consequently, wheat growers need effective crop management strategies to prevent excess free asparagine accumulation in the grain while not compromising other desirable traits, such as crop yield, protein content, disease resistance and

*Correspondence: nigel.halford@rothamsted.ac.uk

¹ Plant Sciences Department, Rothamsted Research, Harpenden AL5 2JQ, UK

Full list of author information is available at the end of the article



© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

stress tolerance. These strategies can only be developed through a greater understanding of the mechanisms and functions underlying asparagine accumulation.

Asparagine in normal development

As a proteinogenic amino acid, asparagine is a structural component of proteins, but its role in plant biology extends beyond this. Asparagine has an essential role in nitrogen storage and transport in many plant species, acting as the main transport molecule of reduced nitrogen in the vasculature; likely because it is the amino acid with the highest nitrogen to carbon ratio (Lea et al. 2007; Gaufichon et al. 2010). Nitrogen is initially absorbed from the soil as ammonium or nitrate, the latter being reduced by nitrate reductase (NR) to form nitrite, which is then reduced by nitrite reductase (NiR) to form ammonium (Masclaux-Daubresse et al. 2010). Assimilation then occurs via the glutamine synthetase (GS)–glutamate synthase (GOGAT) cycle, in which GS incorporates ammonia into glutamine. Finally, asparagine can then be synthesised from glutamine by asparagine synthetase.

Across different plant species, asparagine transport and accumulation dynamics differ widely. In bread wheat (*Triticum aestivum*), the dynamics and magnitude of asparagine transport within the plant are unclear. In particular, it is not known if asparagine is imported into the grain or how much this import, if occurring, contributes to grain asparagine levels relative to in situ asparagine synthesis. Although this has not been studied directly, expression analyses of asparagine synthetase genes highlight in situ synthesis as the foremost determinant, as discussed below. In some species though, asparagine transport enables nitrogen mobilisation from source to sink organs in the xylem and enables nitrogen remobilisation in the phloem, in processes such as seed-filling in *Arabidopsis* (Lam et al. 2003) and leaf senescence (Lea et al. 2007; Herrera-Rodríguez et al. 2006; de Michele et al. 2009). Asparagine also accumulates to higher levels than any other amino acid during germination in many species, but not in others (Lea et al. 2007). This mobilisation and remobilisation of nitrogen in the form of asparagine allows some plant species to transport nitrogen safely, whereas accumulation of excess ammonia, for example, is toxic (Britto et al. 2001). These differences in asparagine transport dynamics have important consequences for crop plants as well: most asparagine in chicory roots, for example, is transported from leaves (Soares 2020), whereas asparagine is synthesised in situ in potato tubers and is not imported from the leaves at all (Chawla et al. 2012; Muttucumararu et al. 2014). Such differences will influence any strategies used to reduce asparagine levels in crop plants. The function of asparagine as a means of nitrogen mobilisation in some plant

species is supported by a number of functional studies on asparagine synthetase genes as well, which also highlight the different roles of these genes and the enzymes they encode (Table 1). Such studies have also suggested that the overexpression of certain endogenous asparagine synthetase genes may be a viable strategy for improving nitrogen use efficiency for some species (reviewed in McAllister et al. (2012)), but the outcome is variable depending on the species and the gene.

Asparagine is synthesised similarly across eukaryotes by the asparagine synthetases, which transfer an amine group from glutamine to aspartate (Lea et al. 2007; Lomelino et al. 2017; Dang et al. 1996). This reaction requires ATP to occur and results in the formation of asparagine and glutamate (Fig. 1). The breakdown of asparagine is controlled by different proteins called asparaginases, which are also present across eukaryotes and catalyse the breakdown of asparagine back into aspartate in a hydrolysis reaction that releases ammonia (Fig. 1) (Lea et al. 2007; Batool et al. 2016). In bread wheat, there are five distinct asparagine synthetase genes: *TaASN1*, *TaASN2*, *TaASN3.1*, *TaASN3.2*, and *TaASN4*, which show different patterns of spatial and temporal expression during development (Xu et al. 2018; Gao et al. 2016). *TaASN1* is the most highly expressed during early development in vegetative tissues, whereas *TaASN2* is the most highly expressed in the embryo and endosperm during late development; both are upregulated in response to sulphur deficiency (Xu et al. 2018; Gao et al. 2016; Curtis et al. 2019). These studies also highlighted that *TaASN3.1*, *TaASN3.2*, and *TaASN4* are all expressed at much lower levels than *TaASN1* and *TaASN2* and these expression patterns are reflected in publicly available RNA-seq datasets (Ramírez-González et al. 2018). At the homoeologue level, *TaASN1* is differentially expressed across the A, B, and D genomes and *TaASN2* is expressed differently across the A and D genomes (Curtis et al. 2019; Ramírez-González et al. 2018). The absence of a B genome *TaASN2* homoeologue in some varieties and its presence in others has previously been noted (Xu et al. 2018), but its origin and effects currently remain unclear.

The wheat asparaginase genes are less well characterised, but there is some information regarding their genetics and expression. Curtis et al. (2019), for example, identified seven putative asparaginase genes in the wheat genome and showed that the expression of one of these was responsive to sulphur deficiency in the embryo and endosperm. In tobacco and lupin, asparaginase gene expression is highest in young developing tissues and in tissues of the developing seed, both of which are nitrogen sinks (Grant and Bevan 1994). Potassium-dependent and potassium-independent forms of asparaginase are also present (Sicciechowicz

Table 1 Functional studies of asparagine synthetase genes (ASN) and the conclusions made from those studies

Species	Modulation	Reported conclusions	Study
<i>Arabidopsis thaliana</i>	ASN1 overexpression	Tolerance to N deprivation in germination Enhanced seed protein content	Lam et al. (2003)
		Slightly more N and protein content Higher dry weight	Gaufichon et al. (2017)
	CaAS1 overexpression	Enhanced disease resistance	Hwang et al. (2011)
	ASN1 knockout	Some disruption of seed formation Slightly less N and more carbon Aberrant cell patterns in the embryo	Gaufichon et al. (2017)
	ASN1 silencing	Negligible effect on virus replication	Fernández-Calvino et al. (2016)
	ASN2 overexpression	Increased asparagine levels	Igarashi et al. (2009)
	ASN2 knockout	Reduced salt tolerance	Maaroufi-Dguimi et al. (2011)
<i>Brassica napus</i> (oilseed rape)	Asna (<i>E. coli</i>) overexpression	Ammonium accumulation and defective growth No visible phenotype in development No difference in seed carbon or N	Gaufichon et al. (2013) Gaufichon et al. (2016)
		Poorer performance at low N application than WT Better performance at high N	Seiffert et al. (2004)
<i>Capsicum annuum</i> (capsicum pepper)	CaAS1 silencing	Increased susceptibility to disease	Hwang et al. (2011)
<i>Lactuca sativa</i> (Garden lettuce)	Asna (<i>E. coli</i>) overexpression	Faster vegetative growth and greater dry weight Improved N status	Giannino et al. (2008)
<i>Nicotiana benthamiana</i>	ASN silencing	Morphological defects upon infection Reduced virus accumulation	Fernández-Calvino et al. (2016)
<i>Oryza sativa</i> (rice)	ASN1 knockout	Reduced stature and fewer tillers	Luo et al. (2019)

N nitrogen

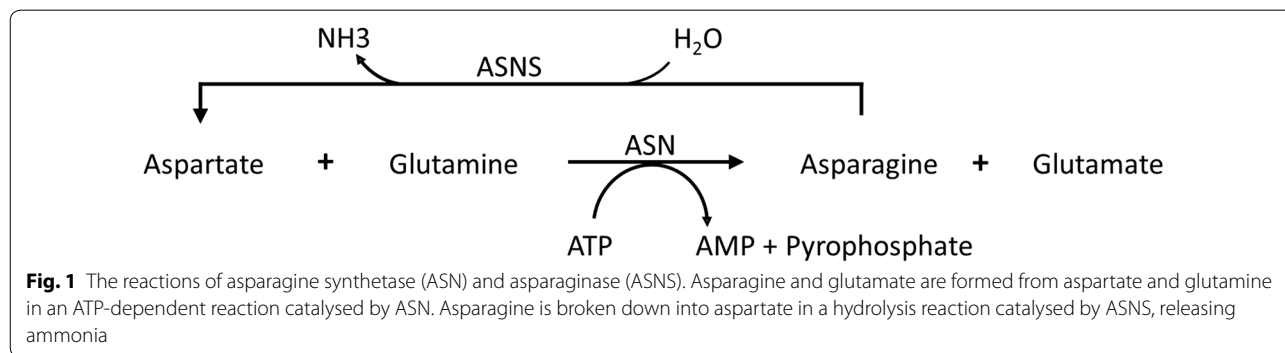


Fig. 1 The reactions of asparagine synthetase (ASN) and asparaginase (ASNS). Asparagine and glutamate are formed from aspartate and glutamine in an ATP-dependent reaction catalysed by ASN. Asparagine is broken down into aspartate in a hydrolysis reaction catalysed by ASNS, releasing ammonia

et al. 1988a), which may be the case in wheat as well. Expression of both potassium-dependent and potassium-independent asparaginases during development in *Arabidopsis* is similarly localised to sink tissues, but the potassium-dependent asparaginase is expressed at lower levels (Bruneau et al. 2006) and asparaginase function is not required for normal development (Ivanov et al. 2012). In contrast, the potassium-dependent asparaginase of *Lotus japonicus* is crucial for plant growth and seed production (Credali et al. 2013). Further analysis of asparaginase gene expression is

required to fully understand the function of asparaginase in wheat and its impact on asparagine levels and nitrogen mobilisation.

Why does asparagine accumulate during stress?

Alongside its role in normal development, asparagine has been shown to accumulate in response to diverse types of abiotic and biotic stressors in many different species; including disease, salt and water stress, and nutrient deficiencies, the latter in particular when nitrogen is plentiful but other minerals are lacking (see Lea et al. (2007),

Stewart and Larher (1980) for reviews). Functional studies have indicated that this stress-induced asparagine accumulation may be adaptive against some stressors such as disease and mineral limitation (Table 1). One possible explanation for this is that asparagine accumulates during stress as part of the nitrogen remobilisation process or to store nitrogen, similarly to its function in normal development (Fig. 2). This has been proposed to happen during infection, in order to divert nitrogen away from the pathogen and to sequester it elsewhere, based on analyses of tomato leaves in response to *Pseudomonas syringae* (Olea et al. 2004). In this model, ammonia released from stress-induced proteolysis is assimilated into glutamine in mesophyll cells. Glutamine is subsequently exported from these cells into the phloem, where asparagine is then synthesised. Asparagine transport then allows mobilisation of nitrogen to healthy tissue and the pathogen is deprived of a source of nitrogen from the host. Hwang et al. (2011) also show

that infection-induced asparagine synthetase expression leads to reactive oxygen species (ROS) bursts and nitric oxide (NO) production, both of which have major roles in defence, so asparagine accumulation may also increase disease resistance this way. Consequently, overexpression of asparagine synthetase genes can confer greater disease resistance, whereas asparagine synthetase gene silencing can confer greater sensitivity (Hwang et al. 2011). However, Fernández-Calvino et al. (2016) show that silencing of asparagine synthetase gene expression in *Nicotiana benthamiana* can cause a reduction in viral replication. They suggest that this is because asparagine accumulation detoxifies the cell of ammonia, allowing the cell to remain healthy and consequently allowing the virus to replicate to a greater extent. Asparagine accumulation has also been recorded in wheat grain in response to disease, specifically in response to the withdrawal of fungicide application (Curtis et al. 2016), indicating that these processes may also occur in wheat.

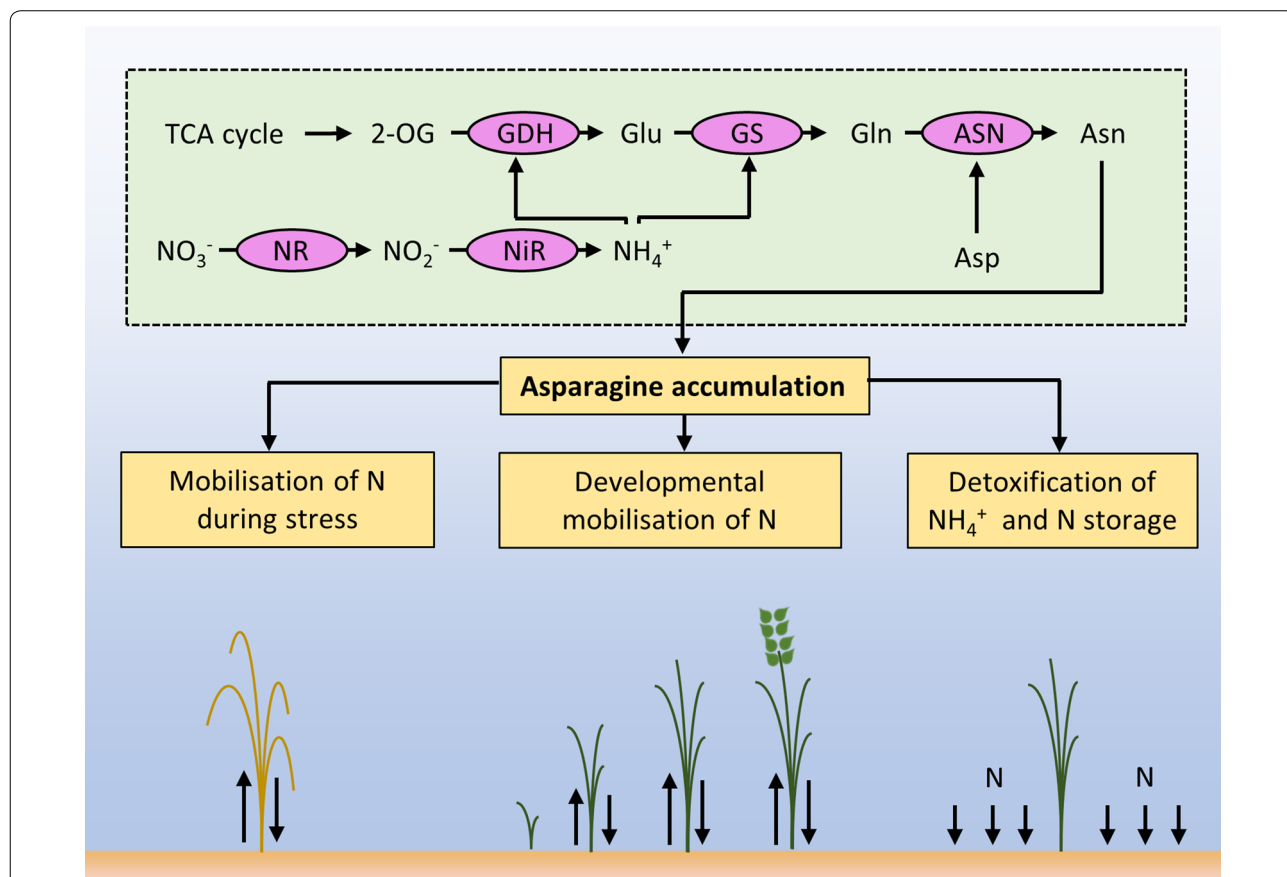


Fig. 2 Synthesis and functions of asparagine. Nitrate (NO_3^-) is absorbed from the soil and reduced to ammonia (NH_4^+) through nitrate reductase (NR) and nitrite reductase (NiR). Ammonia is assimilated via glutamate dehydrogenase (GDH) and glutamine synthetase (GS) to form glutamine (Glu), which is then used to form asparagine (Asn) alongside aspartate (Asp). Asparagine mobilises nitrogen from source to sink tissues during germination, vegetative growth, senescence, and seed filling, as well as during stress. Detoxification of ammonia may also be an important function of asparagine accumulation when nitrogen (N) is abundant and during stress when ammonia accumulates

Asparagine accumulation may also play a role in nitrogen remobilisation and ammonia detoxification during abiotic stress. Beato et al. (2014) suggest that asparagine accumulation occurs for these reasons as a result of a high ammonia to hexose ratio in the cell. Many different abiotic stressors can induce general energy stress (Lastdrager et al. 2014) and proteolysis (Hildebrandt et al. 2015), causing decreases in cellular hexose levels and increases in ammonia. Excess ammonia produced by proteolysis can be recycled into amino acids and asparagine in particular by the GS/GOGAT cycle and asparagine synthetase, respectively, thereby preventing the toxic build-up of ammonia as well. This function of asparagine accumulation remains experimentally unverified, but Lam et al. (2003) have shown that asparagine accumulation is adaptive against nitrogen limitation during germination, and Maaroufi-Dguimi et al. (2011) have also suggested a role in salt tolerance.

The proposed role of asparagine accumulation in salt and drought tolerance is comparable to the role of the amino acid proline. Proline is considered to play a role in stress tolerance against drought and salt stress: its accumulation allows it to function as an osmolyte and prevent ROS bursts, although its adaptive value across species is uncertain (Szabados and Savouré 2010). Rashmi et al. (2019) suggest that asparagine may have a similar role as an osmolyte, because of the upregulation of asparagine synthetase gene expression during salt stress in *Pandanus odorifer*. However, Yadav et al. (2019) demonstrate that asparagine correlates negatively with yield-gap based drought tolerance in wheat. Based on this, they suggest that asparagine instead accumulates as a result of drought-induced senescence and that asparagine accumulation is indicative of poor drought tolerance. Varieties of wheat less tolerant to drought will show more drought-induced senescence, which increases asparagine levels and asparagine synthetase gene expression. Therefore, the function of asparagine accumulation during abiotic stress in wheat may be principally one of nitrogen remobilisation and ammonia detoxification instead.

How does asparagine accumulate during stress?

The accumulation of asparagine during plant stress is reflected in the upregulation of asparagine synthetase genes in response to diverse stressors in a range of plant species. Such upregulation in response to diverse types of stress has been observed, for example, in sunflower (*Helianthus annuus*) (Herrera-Rodríguez et al. 2007), Arabidopsis (*Arabidopsis thaliana*) (Lam et al. 1998; Baena-González et al. 2007), maize (*Zea mays*) (Chevalier et al. 1996), and wheat (*Triticum aestivum*) (Curtis et al. 2019; Wang et al. 2005). Upregulation has also been observed in soybean (*Glycine max*) (Antunes et al. 2008),

sunflower (*Helianthus annuus*) (Herrera-Rodríguez et al. 2004), barley (*Hordeum vulgare*) (Avila-Ospina et al. 2015), poplar (*Populus simonii* × *Populus nigra*) (Qu et al. 2019), and common bean (*Phaseolus vulgaris*) (Osuna et al. 2001) in response to nitrogen. This implies that the asparagine that accumulates during stress is synthesised predominantly de novo and not just as a result of proteolysis and amino acid catabolism. Such studies showing the upregulation of asparagine synthetase gene expression, alongside a comprehensive metabolic network (Curtis et al. 2018a) detailing its regulation in plants, suggest that asparagine synthetase is primarily regulated at the transcriptional level, and therefore that asparagine accumulation is determined in the main by the level of asparagine synthetase gene expression. In the proposed regulatory pathways for the asparagine synthetase genes in Arabidopsis, there are two distinct pathways of upregulation for *AtASN1* and *AtASN2* (Curtis et al. 2018a). For *AtASN1*, the signalling pathway (Fig. 3) starts off with activation of SnRK1.1. and SnRK1.2 (sucrose non-fermenting-1-related protein kinases) in response to low glucose availability and/or the activity of SnAKs (SnRK1-activating kinases). The SnRK1s are protein kinases that are active during periods of low cellular energy status and are involved in many diverse processes, including autophagy, metabolism, and stress responses (Wurzinger et al. 2018; Rodríguez et al. 2019). The SnAKs are mostly known for their ability to regulate the SnRK1s and the downstream processes they control (Glab et al. 2017). The signal is then relayed through basic leucine zipper (bZIP) transcription factors; these are known to regulate a range of developmental and stress responses, often through specific dimerisations with one another (Dröge-Laser and Weiste 2018). Upon activation by the SnRK1s, the bZIPs upregulate *AtASN1* to drive asparagine accumulation.

Studies in Arabidopsis have indicated that regulation of *AtASN1* expression by SnRK1 and bZIP transcription factors is highly responsive to nutrients (Baena-González et al. 2007). This nutrient control is mostly exerted by trehalose-6-phosphate (T6P), which acts as an inhibitor of SnRK1 (Zhang et al. 2009). T6P abundance closely traces the levels of cellular sucrose, so acts as a sugar signal. Consequently, SnRK1 is inhibited under conditions of high sucrose, such as during the dark and under stress (Baena-González et al. 2007). SnRK1 has also been shown to be impacted by stressors that downregulate glucose metabolism. For example, Dong et al. (2017) showed that sulphur deprivation was able to activate SnRK1 through downregulated glucose metabolism and through the reduction in activity of target of rapamycin (TOR). TOR is a protein kinase that, like SnRK1, responds to many different environmental cues

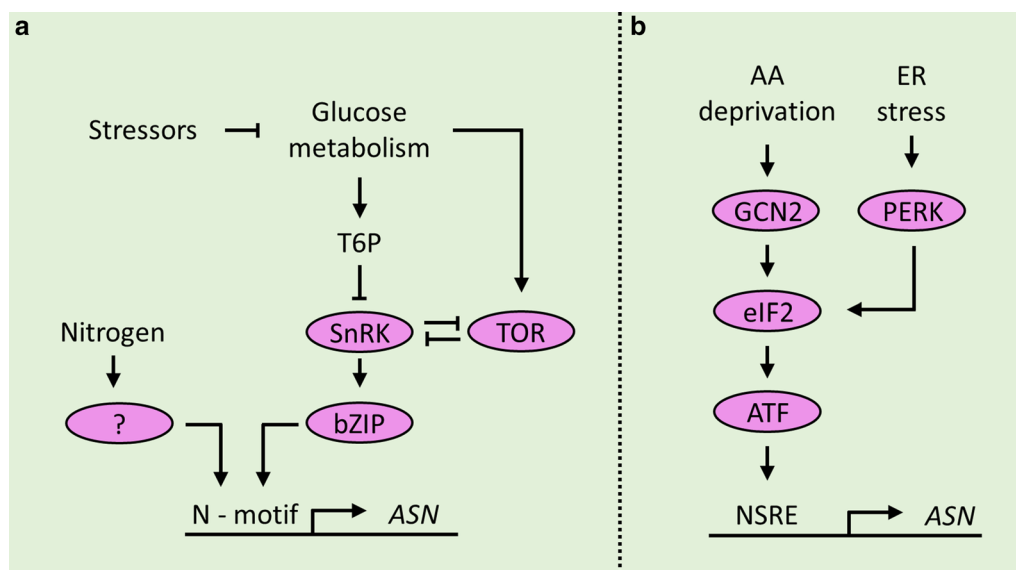


Fig. 3 Models of asparagine synthetase regulation. **a** Regulation of asparagine synthetase (*ASN*) gene expression in plants by stress and nitrogen. **b** Regulation of *ASN* in mammals by amino acid (AA) deprivation and endoplasmic reticulum (ER) stress

to regulate growth and metabolism, but mostly in the opposite direction to SnRK1 (Shi et al. 2018). It is worth noting at this point that the relationship between sucrose and hexoses in different tissues will depend on the activities of the enzymes that interconvert them, such as sucrose synthase, invertase, sucrose phosphate synthase and sucrose phosphate phosphatase. The *Fusarium* toxin deoxynivalenol has also recently been shown to upregulate *TaSnRK1* (Perochon et al. 2019), further suggesting that the responsiveness of SnRK1 to diverse stressors may partially explain the patterns of expression shown by the *ASNs*.

Multiple members of the bZIP family have been shown to activate *AtASNI* (Baena-González et al. 2007; Hanson et al. 2008; Dietrich et al. 2011) and some of these bZIPs possess amino acid motifs that match SnRK1 phosphorylation target sites (Zhang et al. 2008a). Recent experimental evidence has also demonstrated that SnRK1 phosphorylates bZIP63 in vivo (Mair et al. 2015), changing its dimerisation preferences and consequently its activity, confirming direct regulation of bZIPs by SnRK1. Therefore, the asparagine accumulation that occurs in response to sugar deprivation is likely an outcome of signalling through T6P, SnRK1, and bZIP transcription factors, eventually resulting in upregulated *AtASNI* expression.

The promoter region of wheat *TaASNI* also contains a potential regulatory element that is known to be targeted by bZIPs. This element is called the nitrogen (N)—motif, or alternatively a general control nonderepressible-4

(GCN4)—like motif (Albani et al. 1997). bZIPS have also been implicated in the responses observed in wheat grain under sulphur deficiency (Curtis et al. 2019; Raffan et al. 2020), further strengthening the link between stress and asparagine accumulation.

Asparagine accumulation is also likely impacted by the activity and regulation of the asparaginases, although this is less well characterised than asparagine synthetase regulation. Firstly, the activity of asparaginase is responsive to environmental cues. Potassium is necessary in order for some asparaginases to function (Sodek et al. 1980) and the activity of the potassium-dependent asparaginase is negatively regulated by glutamine (Tonin and Sodek 1990). Asparaginase activity is also diurnally regulated, with greater activity in the light during ATP abundance, the glutamate synthase cycle, and protein biosynthesis, and less activity in the dark during proteolysis (Sieciechowicz et al. 1988b, c; Sieciechowicz and Ireland 1989). Secondly, the expression of asparaginase genes in the grain is responsive to the environment, being downregulated by high levels of nitrogen fertiliser (Zheng et al. 2018) or sulphur deficiency (Curtis et al. 2019). The impact of these different environmental cues on asparaginase activity and expression is consistent with the asparagine accumulation that is seen in response to different stressors, suggesting that changes in asparaginase activity could be at least partly responsible for this asparagine accumulation. This is further suggested by functional studies of asparaginase genes, which demonstrate that the absence of asparaginase activity can greatly increase asparagine

accumulation. In *Lotus japonicus*, knockout of asparaginase genes increases asparagine levels massively (Credali et al. 2013), while Arabidopsis mutants lacking asparaginases accumulate more free asparagine in mature seed (Ivanov et al. 2012). Consequently, the asparaginases may also play an important role in controlling stress-induced asparagine accumulation.

Insights on asparagine synthetase regulation from other eukaryotes

The regulation of asparagine synthetase in mammals is, like plants, principally mediated at the transcriptional level. Deprivation of amino acids and glucose has been shown to cause an upregulation of mammalian asparagine synthetase gene expression in many studies (Gong et al. 1991; Guerrini et al. 1993; Hutson and Kilberg 1994; Barbosa-Tessmann et al. 1999a, b; Hutson et al. 1997). The first regulators implicated in this nutritional control of transcription were found to be elements in the promoter of the asparagine synthetase gene, subsequently called nutrient sensing response elements (NSREs) and unfolded protein response (UPR) elements (Guerrini et al. 1993; Barbosa-Tessmann et al. 1999a, 2000) (Fig. 3). These elements were found to be targeted by a combination of upregulating transcription factors, such as activating transcription factors 4 (ATF4) and 5 (ATF5) (Al Sarraj et al. 2005; Siu et al. 2002), alongside downregulating transcription factors, such as activating transcription factor 3 (ATF3), CCAAT-enhancer-binding protein β (C/EBP β), and C/EB homology protein (CHOP) (Chen et al. 2004; Su and Kilberg 2008; Thiaville et al. 2008). The ATFs belong to the mammalian bZIP family and have regulatory roles during development and stress (Hai 2007). The transcription factors CHOP and C/EBP β belong to the CCAAT-enhancer-binding protein (C/EBP) family of transcription factors and also regulate genes involved in growth and metabolism (Nerlov 2007).

ATF4 and ATF5 are regulated to a large extent by eukaryotic translation initiation factor 2 (eIF2), which is in turn regulated by the protein kinases general control nonderepressible (GCN)-2 and protein kinase-like endoplasmic reticulum kinase (PERK) (Balasubramanian et al. 2013). eIF2 is also regulated by RNA-dependent protein kinase (PKR) and haem-regulated inhibitor (HRI), which respond to viral infection and haem deprivation respectively (Wek et al. 2006), but these have not been discussed as much as GCN2 and PERK in the context of asparagine synthetase regulation. GCN2 is activated by uncharged tRNA, which is abundant at low amino acid levels, whereas PERK is activated by endoplasmic reticulum stress (Balasubramanian et al. 2013). Consequently, the signalling pathways controlled by GCN2 and PERK are called the amino acid response (AAR) and unfolded

protein response (UPR), respectively, and they show how nutrient status and stress are linked to asparagine synthetase gene regulation. Interestingly, the SnRK1 homologue, AMP-activated protein kinase (AMPK), does not seem to play a large role in mammalian asparagine synthetase gene regulation: AMPK does not affect asparagine synthetase gene expression during glucose deprivation (Cui et al. 2007), for example, in contrast to the role of SnRK1 in plants described above.

In fungi, GCN2 is activated by diverse stressors such as amino acid limitation, oxidative stress and glucose starvation, and relays this signal by phosphorylating the alpha subunit of eIF2 (eIF2 α). In budding yeast (*Saccharomyces cerevisiae*) this leads to an increase in translation of GCN4, a homologue of ATF4, whilst general protein synthesis is reduced (Mascarenhas et al. 2008; Hinnebusch 1994; Yang et al. 2000). GCN4 binds a “GCN4 box” regulatory motif to induce asparagine synthetase gene expression (Dang et al. 1996; Natarajan et al. 2001). The GCN4 box is analogous to the NSREs in mammals and identical to the putative regulatory motif identified in wheat *TaASN1* described above (Gao et al. 2016). Although plants do not possess a GCN4/ATF homologue (Halford 2005), the nitrogen element may be targeted by other bZIPs instead (Albani et al. 1997), as discussed above. Plants also do not possess *PERK* (Ruberti and Brandizzi 2014), *HRI*, or *PRK* homologues (Halford et al. 2004), but do possess a *GCN2* homologue (Zhang et al. 2003). Like its mammalian and yeast counterparts, plant GCN2 responds to amino acid deprivation (Lageix et al. 2008; Zhang et al. 2008b) and a range of other stressors, including UV-radiation, cold shock, wounding, treatment with methyl jasmonate, salicylate, and cadmium salts (Lageix et al. 2008; Sormani et al. 2011). Plant GCN2 is also activated by interacting with uncharged tRNA, in the same way as the yeast enzyme (Li et al. 2013). However, when *GCN2* is overexpressed in wheat it reduces the expression of *TaASN1* (Byrne et al. 2012), suggesting that it is a negative regulator, so more research is required to elucidate the role of GCN2 in controlling plant asparagine synthetase gene expression. Interestingly, asparagine synthetase forms filaments in yeast during nutrient stress, whereas in mammals the enzymes localise to the mitotic spindle, suggesting “moonlighting” functions distinct from their normal function (Noree et al. 2018). It is not known whether plant asparagine synthetases also show distinct cellular localisations and moonlighting functions.

In recent years, asparagine synthetase regulation in mammals has been of particular interest because of its relevance to cancer biology. The mammalian asparagine synthetase catalyses an identical reaction to the plant asparagine synthetase (Lomelino et al. 2017) and its

basal expression is low in all organs except for the pancreas, where it is important for protecting against nutrient stress (Mukherjee et al. 2020). Asparagine synthetase gene expression in human tumour cells increases in response to glucose deprivation, glutamine deprivation, cisplatin treatment, and hypoxia (Cui et al. 2007; Ameri et al. 2010; Zhang et al. 2014) and appears to play a role in cell proliferation (Gong and Basilico 1990). Notably, Knott et al. (2018) showed that depleting asparagine by dietary restriction or by using asparaginases in a murine model of breast cancer could reduce the number of metastases, the development of which is associated with poorer survival. Asparagine may achieve these effects by acting as an amino acid exchange factor: intracellular asparagine could be exchanged with extracellular amino acids to regulate amino acid homeostasis and metabolism (Krall et al. 2016). Asparagine could also achieve these effects by promoting an epithelial-mesenchymal transition (Knott et al. 2018), a cellular transition associated with increased drug resistance and survivability. The increased understanding of the role of asparagine in cancer (Kanarek et al. 2020; Chiu et al. 2020) has highlighted a need to further assess the role of dietary asparagine in future studies.

There is also a link between GCN2 activity, asparagine synthetase gene expression and sulphur metabolism in mammalian systems, which is intriguing given the response of asparagine synthetase to sulphur deficiency in wheat: both phosphorylation of eIF2 α and expression of asparagine synthetase have been shown to be higher in liver cells of rats fed a diet deficient in sulphur-containing amino acids compared to well-nourished rats (Sikalidis and Stipanuk 2010).

Can asparagine accumulation in wheat be reduced?

Some strategies to reduce asparagine accumulation in wheat cultivation have been published and included in the Acrylamide Toolbox produced by FoodDrinkEurope (2019). The Acrylamide toolbox provides a range of mitigation techniques for all actors in the food supply chain, including the use of asparaginase during processing, changing cooking temperature, excluding overly-browned products after cooking and processing, and the use of crop varieties with low acrylamide-forming potential. In the USA, low asparagine GM potato varieties (Innate[®] and Innate2[®]) have been available for several years (USDA-APHIS 2014), but regulations in the European Union make commercialisation of new GM crops in Europe just about impossible (Halford 2019). No similar GM varieties of wheat or other cereals have been produced, anyway, but there is considerable variation in the basal levels of free asparagine in the grain of conventional wheat varieties (Claus et al. 2006; Taeymans et al.

2004; Curtis et al. 2009, 2018b), suggesting that asparagine levels could be lowered through selection and breeding. However, heritability of free asparagine levels in the grain appears to be low and, as we have discussed already, asparagine accumulation is highly influenced by environmental factors. Consequently, the SNPs and QTL associated with the trait explain only a small proportion of the variance and no common QTL have been identified (Rapp et al. 2018; Emebiri 2014). Asparagine levels for new varieties entering the market are also not required to be published, so it is impossible for the food industry to tell farmers which varieties they would like them to grow. In addition, food businesses generally purchase wheat grain from the world market rather than local suppliers. This ensures that they get the best price available but means that they have little control over the varieties that are grown. As a result, recommended practices for asparagine reduction in wheat currently emphasise crop management strategies (FoodDrinkEurope 2019), specifically the application of nitrogen fertiliser at the minimum levels to ensure that the optimum yield and required protein content is achieved, the application of sulphur fertiliser to ensure that sulphur deficiency is avoided, and the use of fungicides to control disease.

Application of nitrogen fertiliser is known to increase grain asparagine levels across wheat varieties (Claus et al. 2006; Martinek et al. 2009; Weber et al. 2008), reflecting the upregulation of asparagine synthetase gene expression in response to nitrogen seen in many plant species. Application of excess nitrogen fertiliser compromises food safety this way, as well as negatively impacting many aspects of environmental health (Cameron et al. 2013), but is also necessary to achieve good yields to ensure food security and to produce protein levels necessary for breadmaking (Weber et al. 2008). Its application in both conventional and organic farming systems is associated with higher levels of free asparagine in the grain (Stockmann et al. 2018) but organic methods have been shown to achieve lower asparagine levels than conventional farming (Stockmann et al. 2019), likely due to the slower release of nitrogen in that system. Organic systems are also associated with a reduced yield and lower protein content (Stockmann et al. 2018), but the association between protein content and asparagine levels is not strong, indicating that asparagine levels could be reduced through decreased nitrogen application without compromising protein levels too much, at least in some varieties. Martinek et al. (2009) similarly found strong correlations between protein content and asparagine levels in some varieties but not in others, further emphasising the untapped potential of variety selection. Nitrogen application rates of 180 kg per hectare

have been described as enabling accumulation of protein to levels sufficient for breadmaking (Weber et al. 2008) whilst minimising asparagine levels (Stockmann et al. 2018), so this application rate may be optimal for some varieties. Nevertheless, varietal differences in the response to nitrogen application in terms of protein and asparagine accumulation point to a need for asparagine screening in plant breeding programmes in order to develop reliable and useful recommendations.

Adequate sulphur application is another important part of a fertiliser regime in order to reduce free asparagine levels in wheat, as increasing application rates are known to reduce asparagine accumulation (Curtis et al. 2018b; Granvogel et al. 2007). In fact, sulphur deficiency can cause a many-fold increase in free asparagine concentration, and a spike in free asparagine levels in the grain used by a food business is one of the issues most likely to result in the acrylamide levels of a food product becoming unacceptably high (reviewed in Raffan et al. (2020)). As a result, we recommend the application of sulphur at a rate of 20 kg per hectare to all wheat destined for human consumption (Raffan et al. 2020). Sulphur deficiency may increase asparagine levels as a result of increased proteolysis and energy stress, in order to mobilise nitrogen and detoxify ammonia as outlined above (Beato et al. 2014; Dong et al. 2017), or to store nitrogen for synthesis of sulphur-rich proteins when sulphur availability increases (Zhao et al. 1999). Similarly to the relationship between nitrogen and asparagine, the effect of sulphur on asparagine levels also varies greatly depending on the wheat genotype (Curtis et al. 2018b).

The final recommendation is the application of fungicide and good phytosanitary practices, i.e. disease control (FoodDrinkEurope 2019). Application of fungicides has been shown to reduce asparagine accumulation in wheat (Curtis et al. 2016; Martinek et al. 2009), reflecting the effect of disease in general on asparagine accumulation. The response of asparagine levels to fungicide application differs greatly between genotypes (Curtis et al. 2016), likely as a result of different levels of disease resistance, indicating again the potential for variety selection to help control asparagine levels.

These crop management strategies help to address some of the agronomic causes of increases in free asparagine levels, but, as discussed above, research indicates that free asparagine accumulation in wheat grain probably occurs in response to many other stressors as well. Drought, for example, can cause an increase in free asparagine in potato, but the responses are, again, genotype-dependent and complex, with moderate drought stress in potato actually decreasing acrylamide-forming potential in some varieties (Muttucumararu et al. 2015). In wheat, drought stress is known to increase asparagine

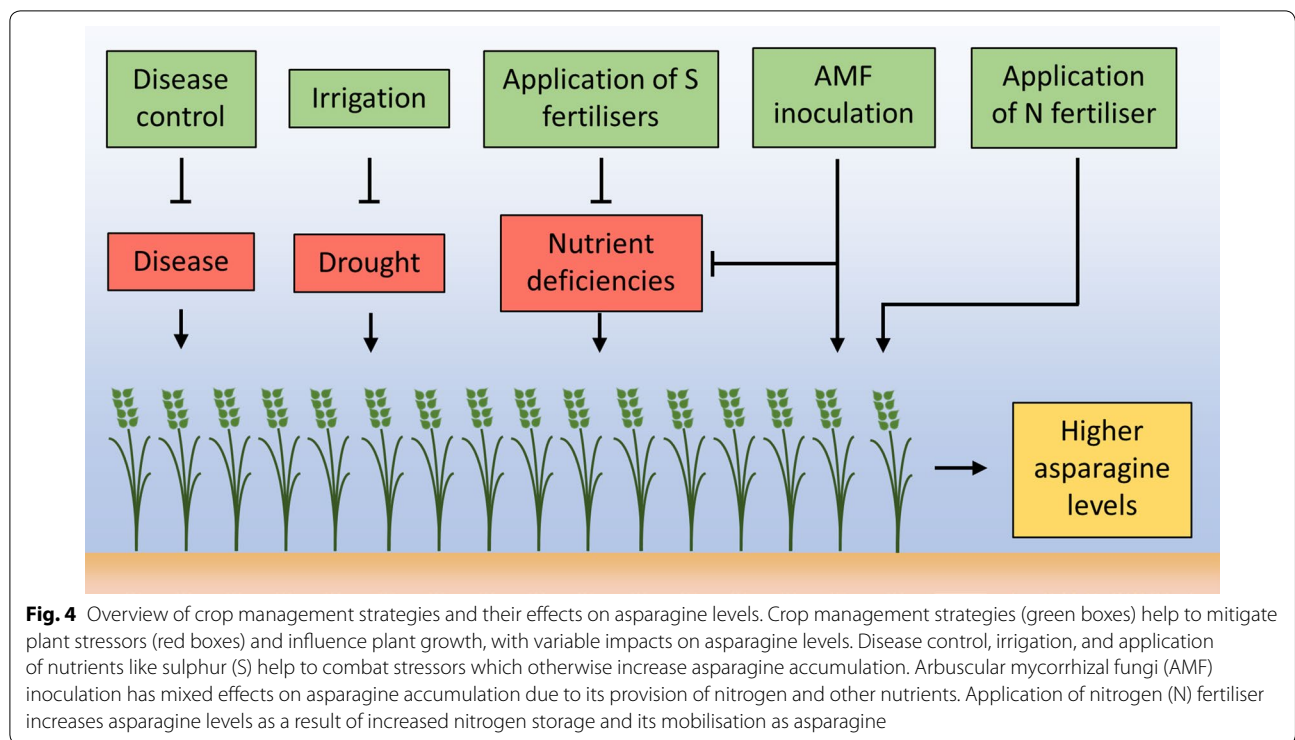
levels (Carillo et al. 2005), which Yadav et al. (2019) attribute to senescence during severe stress.

Deficient or excessive levels of macro- and micro-nutrients can lead to asparagine accumulation in many plant species as well (see Lea et al. (2007), Stewart and Larher (1980) for review) but the effects of precise application rates on asparagine levels in wheat have not been tested. Nutrient availability is significantly altered by arbuscular mycorrhizal fungi (AMF), leading to increases in asparagine levels with greater AMF colonisation in some experiments (Whiteside et al. 2012; Salvioli et al. 2012; Gaude et al. 2015), whilst leading to decreases in others (Saia et al. 2015). Provision of nutrients such as phosphorus by AMF reduces asparagine levels under nitrogen deficiency (Saia et al. 2015) but, under conditions of nitrogen sufficiency, this is likely to be counterbalanced by the effect of increased nitrogen availability. Consequently, different AMF soil inoculation strategies may be able to modulate asparagine levels, and the effectiveness of such an approach could be enhanced if it were used alongside the other crop management strategies described above.

Overall, crop management strategies are crucial in ensuring that free asparagine levels are kept as low as possible (Fig. 4). Given the importance of genotype in determining the magnitude of free asparagine accumulation in response to different crop management strategies, variety selection and breeding, not only for low free asparagine levels per se but also for the interaction between genotype and crop management, is likely to be important as well, if suitable genetic markers can be found.

Conclusions

Attempts to reduce free asparagine levels in wheat grain, and thereby dietary acrylamide intake, must be balanced against other aspects of wheat cultivation and strategies to improve public health. For example, asparagine concentration could be greatly reduced by withholding all nitrogen application, but this would devastate yields and grain protein composition (Hawkesford 2014). Dietary acrylamide intake could also be reduced by less consumption of wholegrain foods (Raffan and Halford 2019), but these foods are generally high in fibre, which is known to decrease chronic inflammation, thereby reducing the risk of cancers, heart disease, and many other health issues (Swann et al. 2019). With regards to flavour, the Maillard reaction is responsible for the formation of many desirable flavour compounds alongside acrylamide (Raffan and Halford 2019), so total reduction of the Maillard reaction would negatively impact flavour as well. Consequently, strategies to reduce asparagine in wheat and acrylamide formation in baked wheat products must account for such outcomes.



Fortunately, the reduction of asparagine in wheat is likely to go hand-in-hand with the reduction of plant stress, which is desirable for all in the food supply chain, and is unlikely to impact flavour formation as much as other acrylamide mitigation strategies (Xu et al. 2016). While the molecular mechanisms linking free asparagine accumulation in wheat grain and nutrient availability have been elucidated in part, the effects of other abiotic stresses remain mostly unknown, although research from other plants and other eukaryotes have provided insights, which we have summarised in this review. Similarly, the function of free asparagine accumulation in wheat has mostly been inferred from its function in other plants. A greater understanding of the functions and mechanisms of asparagine accumulation in wheat will therefore enable the development of new strategies for asparagine reduction, and perhaps the breeding of reliably low asparagine varieties.

Acknowledgements

The authors have no additional acknowledgements.

Authors' contributions

All authors contributed to the writing of this review. All authors read and approved the final manuscript.

Funding

Joseph Oddy is supported by a Biotechnology and Biological Sciences Research Council (BBSRC) Collaborative Training Partnership Studentship in partnership with University of Reading and Mondelez UK R&D Ltd. Sarah Raffan is supported by a BBSRC SWBio iCASE DTP Studentship, with partners:

University of Bristol, AHDB, KWS UK Ltd, Saaten Union UK Ltd, RAGT Seeds Ltd, Syngenta UK Ltd, and Limagrain UK Ltd. Nigel Halford and Mark Wilkinson are supported at Rothamsted Research by the BBSRC via the Designing Future Wheat Programme (BB/P016855/1). BBSRC is part of UK Research and Innovation.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The funders listed above had no role in the composition or writing of the manuscript, or in the decision to publish.

Author details

¹ Plant Sciences Department, Rothamsted Research, Harpenden AL5 2JQ, UK. ² Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK.

Received: 8 June 2020 Accepted: 17 July 2020

Published online: 28 July 2020

References

Al Sarraj J, Vinson C, Thiel G. Regulation of asparagine synthetase gene transcription by the basic region leucine zipper transcription factors ATF5 and CHOP. *Biol Chem*. 2005. <https://doi.org/10.1515/bc.2005.102>.
 Albani D, Hammond-Kosack MCU, Smith C, Conlan S, Colot V, Holdsworth M, Bevan MW. The wheat transcriptional activator SPA: a seed-specific bZIP protein that recognizes the GCN4-like motif in the bifactorial

- endosperm box of prolamin genes. *Plant Cell*. 1997. <https://doi.org/10.1105/tpc.92.171>.
- Ameri K, Luong R, Zhang H, Powell AA, Montgomery KD, Espinosa I, Bouley DM, Harris AL, Jeffrey SS. Circulating tumour cells demonstrate an altered response to hypoxia and an aggressive phenotype. *Br J Cancer*. 2010. <https://doi.org/10.1038/sj.bjc.6605491>.
- Antunes F, Aguilar M, Pineda M, Sodek L. Nitrogen stress and the expression of asparagine synthetase in roots and nodules of soybean (*Glycine max*). *Physiol Plant*. 2008;133:736–43.
- Avila-Ospina L, Marmagne A, Talbot J, Krupinska K, Masclaux-Daubresse C. The identification of new cytosolic glutamine synthetase and asparagine synthetase genes in barley (*Hordeum vulgare* L.), and their expression during leaf senescence. *J Exp Bot*. 2015;66:2013–26.
- Baena-González E, Rolland F, Thevelein JM, Sheen J. A central integrator of transcription networks in plant stress and energy signalling. *Nature*. 2007;448:938–42.
- Balasubramanian MN, Butterworth EA, Kilberg MS. Asparagine synthetase: regulation by cell stress and involvement in tumor biology. *Am J Physiol Endocrinol Metab*. 2013. <https://doi.org/10.1152/ajpen.00015.2013>.
- Barbosa-Tessmann IP, Chen C, Zhong C, Schuster SM, Nick HS, Kilberg MS. Activation of the unfolded protein response pathway induces human asparagine synthetase gene expression. *J Biol Chem*. 1999a. <https://doi.org/10.1074/jbc.274.44.31139>.
- Barbosa-Tessmann IP, Pineda VL, Nick HS, Schuster SM, Kilberg MS. Transcriptional regulation of the human asparagine synthetase gene by carbohydrate availability. *Biochem J*. 1999b. <https://doi.org/10.1042/0264-6021.3390151>.
- Barbosa-Tessmann IP, Chen C, Zhong C, Siu F, Schuster SM, Nick HS, Kilberg MS. Activation of the human asparagine synthetase gene by the amino acid response and the endoplasmic reticulum stress response pathways occurs by common genomic elements. *J Biol Chem*. 2000. <https://doi.org/10.1074/jbc.M000004200>.
- Batool T, Makky EA, Jalal M, Yusoff MM. A comprehensive review on L-asparaginase and its applications. *Appl Biochem Biotechnol*. 2016. <https://doi.org/10.1007/s12010-015-1917-3>.
- Beato VM, Rexch J, Navarro-Gochicoa MT, Camacho-Cristóbal JJ, Herrera-Rodríguez MB, González-Fontes A. Boron deficiency increases expressions of asparagine synthetase, glutamate dehydrogenase and glutamine synthetase genes in tobacco roots irrespective of the nitrogen source. *Soil Sci Plant Nutr*. 2014;60:314–24.
- Britto DT, Siddiqi MY, Glass ADM, Kronzucker HJ. Futile transmembrane NH₄⁺ cycling: a cellular hypothesis to explain ammonium toxicity in plants. *Proc Natl Acad Sci USA*. 2001. <https://doi.org/10.1073/pnas.061034698>.
- Bruneau L, Chapman R, Marsolais F. Co-occurrence of both L-asparaginase subtypes in Arabidopsis: At3g16150 encodes a K⁺-dependent L-asparaginase. *Planta*. 2006. <https://doi.org/10.1007/s00425-006-0245-9>.
- Byrne EH, Prosser I, Muttucumaru N, Curtis TY, Wingler A, Powers S, Halford NG. Overexpression of GCN2-type protein kinase in wheat has profound effects on free amino acid concentration and gene expression. *Plant Biotechnol J*. 2012. <https://doi.org/10.1111/j.1467-7652.2011.00665.x>.
- Cameron KC, Di HJ, Moir JL. Nitrogen losses from the soil/plant system: a review. *Ann Appl Biol*. 2013. <https://doi.org/10.1111/aab.12014>.
- Carillo P, Mastrodonato G, Nacca F, Fuggi A. Nitrate reductase in durum wheat seedlings as affected by nitrate nutrition and salinity. *Funct Plant Biol*. 2005;32:209–19.
- Chawla R, Shakya R, Rommens CM. Tuber-specific silencing of asparagine synthetase-1 reduces the acrylamide-forming potential of potatoes grown in the field without affecting tuber shape and yield. *Plant Biotechnol J*. 2012. <https://doi.org/10.1111/j.1467-7652.2012.00720.x>.
- Chen H, Pan YX, Dudenhausen EE, Kilberg MS. Amino acid deprivation induces the transcription rate of the human asparagine synthetase gene through a timed program of expression and promoter binding of nutrient-responsive basic region/leucine zipper transcription factors as well as localized histone. *J Biol Chem*. 2004. <https://doi.org/10.1074/jbc.M409173200>.
- Chevalier C, Bourgeois E, Just D, Raymond P. Metabolic regulation of asparagine synthetase gene expression in maize (*Zea mays* L.) root tips. *Plant J*. 1996;9:1–11.
- Chiu M, Taurino G, Bianchi MG, Kilberg MS, Bussolati O. Asparagine synthetase in cancer: beyond acute lymphoblastic leukemia. *Front Oncol*. 2020;9:1480.
- Claus A, Schreier P, Weber A, Graeff S, Herrmann W, Claupein W, Schieber A, Carle R. Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads. *J Agric Food Chem*. 2006. <https://doi.org/10.1021/jf061936f>.
- Credali A, García-Calderón M, Dam S, Perry J, Díaz-Quintana A, Parniske M, Wang TL, Stougaard J, Vega JM, Márquez AJ. The K⁺-dependent asparaginase, NSE1, is crucial for plant growth and seed production in *Lotus japonicus*. *Plant Cell Physiol*. 2013. <https://doi.org/10.1093/pcp/pcs156>.
- Cui H, Darmanin S, Natsuisaka M, et al. Enhanced expression of asparagine synthetase under glucose-deprived conditions protects pancreatic cancer cells from apoptosis induced by glucose deprivation and cisplatin. *Cancer Res*. 2007. <https://doi.org/10.1158/0008-5472.CAN-06-2519>.
- Curtis TY, Muttucumaru N, Shewry PR, Parry MAJ, Powers SJ, Elmore JS, Mottram DS, Hook S, Halford NG. Effects of genotype and environment on free amino acid levels in wheat grain: implications for acrylamide formation during processing. *J Agric Food Chem*. 2009. <https://doi.org/10.1021/jf8031292>.
- Curtis TY, Powers SJ, Halford NG. Effects of fungicide treatment on free amino acid concentration and acrylamide-forming potential in wheat. *J Agric Food Chem*. 2016;64:9689–96.
- Curtis TY, Bo V, Tucker A, Halford NG. Construction of a network describing asparagine metabolism in plants and its application to the identification of genes affecting asparagine metabolism in wheat under drought and nutritional stress. *Food Energy Sec*. 2018a. <https://doi.org/10.1002/fes3.126>.
- Curtis TY, Powers SJ, Wang R, Halford NG. Effects of variety, year of cultivation and sulphur supply on the accumulation of free asparagine in the grain of commercial wheat varieties. *Food Chem*. 2018b. <https://doi.org/10.1016/j.foodchem.2017.06.113>.
- Curtis TY, Raffan S, Wan Y, King R, Gonzalez-Urriarte A, Halford NG. Contrasting gene expression patterns in grain of high and low asparagine wheat genotypes in response to sulphur supply. *BMC Genomics*. 2019;20:628.
- Dang VD, Valens M, Bolotin-Fukuhara M, Daignan-Fornier B. Cloning of the *ASN1* and *ASN2* genes encoding asparagine synthetases in *Saccharomyces cerevisiae*: differential regulation by the CCAAT-box-binding factor. *Mol Microbiol*. 1996. <https://doi.org/10.1046/j.1365-2958.1996.d01-1715.x>.
- de Michele R, Formentin E, Todesco M, et al. Transcriptome analysis of Medicago truncatula leaf senescence: similarities and differences in metabolic and transcriptional regulations as compared with Arabidopsis, nodule senescence and nitric oxide signalling. *New Phytol*. 2009. <https://doi.org/10.1111/j.1469-8137.2008.02684.x>.
- Dietrich K, Weltmeier F, Ehlert A, Weiste C, Stahl M, Harter K, Dröge-Lasera W. Heterodimers of the Arabidopsis transcription factors bZIP1 and bZIP53 reprogram amino acid metabolism during low energy stress. *Plant Cell*. 2011. <https://doi.org/10.1105/tpc.110.075390>.
- Dong Y, Silbermann M, Speiser A, et al. Sulfur availability regulates plant growth via glucose-TOR signaling. *Nat Commun*. 2017. <https://doi.org/10.1038/s41467-017-01224-w>.
- Dröge-Lasera W, Weiste C. The C/S1 bZIP network: a regulatory hub orchestrating plant energy homeostasis. *Trends Plant Sci*. 2018. <https://doi.org/10.1016/j.tplants.2018.02.003>.
- Emebiri LC. Genetic variation and possible SNP markers for breeding wheat with low-grain asparagine, the major precursor for acrylamide formation in heat-processed products. *J Sci Food Agric*. 2014. <https://doi.org/10.1002/jsfa.6434>.
- European Commission. Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and Benchmark Levels for the reduction of the presence of acrylamide in food. Brussels: European Commission; 2017.
- Fernández-Calvino L, Guzmán-Benito I, del Toro FJ, Donaire L, Castro-Sanz AB, Ruiz-Ferrer V, Llavec C. Activation of senescence-associated Dark-inducible (DIN) genes during infection contributes to enhanced susceptibility to plant viruses. *Mol Plant Pathol*. 2016. <https://doi.org/10.1111/mpp.12257>.
- FoodDrinkEurope. Acrylamide toolbox 2019. Brussels: Food Drink Europe; 2019.

- Gao R, Curtis TY, Powers SJ, Xu H, Huang J, Halford NG. Food safety: structure and expression of the asparagine synthetase gene family of wheat. *J Cereal Sci.* 2016. <https://doi.org/10.1016/j.jcs.2016.01.010>.
- Gaude N, Bortfeld S, Erban A, Kopka J, Krajinski F. Symbiosis dependent accumulation of primary metabolites in arbuscule-containing cells. *BMC Plant Biol.* 2015. <https://doi.org/10.1186/s12870-015-0601-7>.
- Gaufichon L, Reisdorf-Cren M, Rothstein SJ, Chardon F, Suzuki A. Biological functions of asparagine synthetase in plants. *Plant Sci.* 2010. <https://doi.org/10.1016/j.plantsci.2010.04.010>.
- Gaufichon L, Masclaux-Daubresse C, Tcherkez G, Reisdorf-Cren M, Sakakibara Y, Hase T, Clement G, Avice J, Grandjean O, Marmagne A. *Arabidopsis thaliana* *ASN2* encoding asparagine synthetase is involved in the control of nitrogen assimilation and export during vegetative growth. *Plant Cell Environ.* 2013;36:328–42.
- Gaufichon L, Marmagne A, Yoneyama T, Hase T, Clément G, Trassaert M, Xu X, Shakibaei M, Najihi A, Suzuki A. Impact of the disruption of *ASN3*-encoding asparagine synthetase on *Arabidopsis* development. *Agronomy.* 2016;6:12.
- Gaufichon L, Marmagne A, Belcram K, Yoneyama T, Sakakibara Y, Hase T, Grandjean O, Clément G, Citerne S, Boutet-Mercey S. *ASN 1*-encoded asparagine synthetase in floral organs contributes to nitrogen filling in *Arabidopsis* seeds. *Plant J.* 2017;91:371–93.
- Giannino D, Nicolodi C, Testone G, Frugis G, Pace E, Santamaria P, Guardasole M, Mariotti D. The overexpression of asparagine synthetase a from *E. coli* affects the nitrogen status in leaves of lettuce (*Lactuca sativa* L.) and enhances vegetative growth. *Euphytica.* 2008. <https://doi.org/10.1007/s10681-007-9506-3>.
- Glab N, Oury C, Guérinier T, Domenichini S, Crozet P, Thomas M, Vidal J, Hodges M. The impact of *Arabidopsis thaliana* SNF1-related-kinase 1 (SnRK1)-activating kinase 1 (SnAK1) and SnAK2 on SnRK1 phosphorylation status: characterization of a SnAK double mutant. *Plant J.* 2017. <https://doi.org/10.1111/tpj.13445>.
- Gong SS, Basilico C. A mammalian temperature-sensitive mutation affecting G1 progression results from a single amino acid substitution in asparagine synthetase. *Nucl Acids Res.* 1990. <https://doi.org/10.1093/nar/18.12.3509>.
- Gong SS, Guerrini L, Basilico C. Regulation of asparagine synthetase gene expression by amino acid starvation. *Mol Cell Biol.* 1991. <https://doi.org/10.1128/mcb.11.12.6059>.
- Grant M, Bevan MW. Asparaginase gene expression is regulated in a complex spatial and temporal pattern in nitrogen-sink tissues. *Plant J.* 1994. <https://doi.org/10.1111/j.1365-313X.1994.00695.x>.
- Granvogel M, Wieser H, Koehler P, von Tucher S, Schieberle P. Influence of sulfur fertilization on the amounts of free amino acids in wheat. Correlation with baking properties as well as with 3-aminopropionamide and acrylamide generation during baking. *J Agric Food Chem.* 2007;55:4271–7.
- Guerrini L, Gong SS, Mangasarian K, Basilico C. Cis- and trans-acting elements involved in amino acid regulation of asparagine synthetase gene expression. *Mol Cell Biol.* 1993. <https://doi.org/10.1128/mcb.13.6.3202>.
- Hai T. The ATF transcription factors in cellular adaptive responses. In: Ma J, editor. *Gene expression and regulation*. New York: Springer; 2007. https://doi.org/10.1007/978-0-387-40049-5_20.
- Halford NG. Regulation of carbon and amino acid metabolism: roles of sucrose nonfermenting-1-related protein kinase-1 and general control nonderepressible-2-related protein kinase. *Adv Bot Res.* 2005. [https://doi.org/10.1016/S0065-2296\(05\)43002-5](https://doi.org/10.1016/S0065-2296(05)43002-5).
- Halford NG. Legislation governing genetically modified and genome-edited crops in Europe: the need for change. *J Sci Food Agric.* 2019. <https://doi.org/10.1002/jsfa.9227>.
- Halford NG, Hey S, Jhurrea D, Laurie S, McKibbin RS, Zhang Y, Paul MJ. Highly conserved protein kinases involved in the regulation of carbon and amino acid metabolism. *J Exp Bot.* 2004. <https://doi.org/10.1093/jxb/erh019>.
- Hanson J, Hanssen M, Wiese A, Hendriks MMWB, Smeekens S. The sucrose regulated transcription factor bZIP11 affects amino acid metabolism by regulating the expression of *ASPARAGINE SYNTHETASE1* and *PROLINE DEHYDROGENASE2*. *Plant J.* 2008. <https://doi.org/10.1111/j.1365-313X.2007.03385.x>.
- Hawkesford MJ. Reducing the reliance on nitrogen fertilizer for wheat production. *J Cereal Sci.* 2014. <https://doi.org/10.1016/j.jcs.2013.12.001>.
- Herrera-Rodríguez MB, Maldonado JM, Pérez-Vicente R. Light and metabolic regulation of *HAS1*, *HAS1.1* and *HAS2*, three asparagine synthetase genes in *Helianthus annuus*. *Plant Physiol Biochem.* 2004;42:511–8.
- Herrera-Rodríguez MB, Maldonado JM, Pérez-Vicente R. Role of asparagine and asparagine synthetase genes in sunflower (*Helianthus annuus*) germination and natural senescence. *J Plant Physiol.* 2006. <https://doi.org/10.1016/j.jplph.2005.10.012>.
- Herrera-Rodríguez MB, Perez-Vicente R, Maldonado JM. Expression of asparagine synthetase genes in sunflower (*Helianthus annuus*) under various environmental stresses. *Plant Physiol Biochem.* 2007;45:33–8.
- Hildebrandt TM, Nunes Nesi A, Araújo WL, Braun HP. Amino acid catabolism in plants. *Mol Plant.* 2015. <https://doi.org/10.1016/j.molp.2015.09.005>.
- Hinnebusch AG. Translational control of GCN4: an in vivo barometer of initiation-factor activity. *Trends Biochem Sci.* 1994. [https://doi.org/10.1016/0968-0004\(94\)90089-2](https://doi.org/10.1016/0968-0004(94)90089-2).
- Hutson RG, Kilberg MS. Cloning of rat asparagine synthetase and specificity of the amino acid-dependent control of its mRNA content. *Biochem J.* 1994. <https://doi.org/10.1042/bj3040745>.
- Hutson RG, Kitoh T, Moraga Amador DA, Cosic S, Schuster SM, Kilberg MS. Amino acid control of asparagine synthetase: relation to asparaginase resistance in human leukemia cells. *Am J Physiol.* 1997. <https://doi.org/10.1152/ajpcell.1997.272.5.c1691>.
- Hwang IS, An SH, Hwang BK. Pepper *asparagine synthetase 1* (*CaAS1*) is required for plant nitrogen assimilation and defense responses to microbial pathogens. *Plant J.* 2011;67:749–62.
- Igarashi D, Ishizaki T, Totsuka K, Ohsumi C. *ASN2* is a key enzyme in asparagine biosynthesis under ammonium sufficient conditions. *Plant Biotechnol.* 2009. <https://doi.org/10.5511/plantbiotechnology.26.153>.
- Ivanov A, Kameka A, Pajak A, Bruneau L, Beyaert R, Hernández-Sebastià C, Marsolais F. *Arabidopsis* mutants lacking asparaginases develop normally but exhibit enhanced root inhibition by exogenous asparagine. *Amino Acids.* 2012. <https://doi.org/10.1007/s00726-011-0973-4>.
- Kanarek N, Petrova B, Sabatini DM. Dietary modifications for enhanced cancer therapy. *Nature.* 2020;579:507–17.
- Knott SRV, Wagenblast E, Khan S, et al. Asparagine bioavailability governs metastasis in a model of breast cancer. *Nature.* 2018. <https://doi.org/10.1038/nature25465>.
- Krall AS, Xu S, Graeber TG, Braas D, Christofk HR. Asparagine promotes cancer cell proliferation through use as an amino acid exchange factor. *Nat Commun.* 2016. <https://doi.org/10.1038/ncomms11457>.
- Lageix S, Lanet E, Pouch-Pélessier MN, Espagnol MC, Robaglia C, Deragon JM, Pélessier T. *Arabidopsis* eIF2a kinase GCN2 is essential for growth in stress conditions and is activated by wounding. *BMC Plant Biol.* 2008. <https://doi.org/10.1186/1471-2229-8-134>.
- Lam H, Hsieh M, Coruzzi G. Reciprocal regulation of distinct asparagine synthetase genes by light and metabolites in *Arabidopsis thaliana*. *Plant J.* 1998;16:345–53.
- Lam HM, Wong P, Chan HK, Yam KM, Chen L, Chow CM, Coruzzi GM. Overexpression of the *ASN1* gene enhances nitrogen status in seeds of *Arabidopsis*. *Plant Physiol.* 2003;132:926–35.
- Lastdrager J, Hanson J, Smeekens S. Sugar signals and the control of plant growth and development. *J Exp Bot.* 2014. <https://doi.org/10.1093/jxb/ert474>.
- Lea PJ, Sodek L, Parry MAJ, Shewry R, Halford NG. Asparagine in plants. *Ann Appl Biol.* 2007;150:1–26.
- Li MW, Auyeung WK, Lam HM. The GCN2 homologue in *Arabidopsis thaliana* interacts with uncharged tRNA and uses *Arabidopsis* eIF2a molecules as direct substrates. *Plant Biol.* 2013. <https://doi.org/10.1111/j.1438-8677.2012.00606.x>.
- Lomelino CL, Andring JT, McKenna R, Kilberg MS. Asparagine synthetase: function, structure, and role in disease. *J Biol Chem.* 2017. <https://doi.org/10.1074/jbc.R117.819060>.
- Luo L, Qin RY, Liu T, Yu M, Yang TW, Xu GH. OsASN1 plays a critical role in asparagine-dependent rice development. *Int J Mol Sci.* 2019. <https://doi.org/10.3390/ijms20010130>.
- Maaroufi-Dguimi H, Debouba M, Gaufichon L, Clément G, Gouia H, Hajjaji A, Suzuki A. An *Arabidopsis* mutant disrupted in *ASN2* encoding asparagine synthetase 2 exhibits low salt stress tolerance. *Plant Physiol Biochem.* 2011;49:623–8.

- Mair A, Pedrotti L, Wurzinger B, et al. SnRK1-triggered switch of bZIP63 dimerization mediates the low-energy response in plants. *eLife*. 2015. <https://doi.org/10.7554/eLife.05828>.
- Martinek P, Klem K, Váňová M, Bartáčková V, Večerková L, Bucher P, Hajšlová J. Effects of nitrogen nutrition, fungicide treatment and wheat genotype on free asparagine and reducing sugars content as precursors of acrylamide formation in bread. *Plant Soil Environ*. 2009. <https://doi.org/10.17221/382-pse>.
- Mascarenhas C, Edwards-Ingram LC, Zeef L, Shenton D, Ashe MP, Grant CM. Gcn4 is required for the response to peroxide stress in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell*. 2008. <https://doi.org/10.1091/mbc.E07-11-1173>.
- Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Ann Bot*. 2010. <https://doi.org/10.1093/aob/mcq028>.
- McAllister CH, Beatty PH, Good AG. Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnol J*. 2012. <https://doi.org/10.1111/j.1467-7652.2012.00700.x>.
- Mottram DS, Wedzicha BL, Dodson AT. Food chemistry: acrylamide is formed in the Maillard reaction. *Nature*. 2002. <https://doi.org/10.1038/419448a>.
- Mukherjee A, Ahmed N, Rose FT, Ahmad AN, Javed TA, Wen L, Bottino R, Xiao X, Kilberg MS, Husain SZ. Asparagine synthetase is highly expressed at baseline in the pancreas through heightened PERK signaling. *Cell Mol Gastroenterol*. 2020. <https://doi.org/10.1016/j.jcmgh.2019.08.003>.
- Muttucumaru N, Keys AJ, Parry MAJ, Powers SJ, Halford NG. Photosynthetic assimilation of ^{14}C into amino acids in potato (*Solanum tuberosum*) and asparagine in the tubers. *Planta*. 2014. <https://doi.org/10.1007/s00425-013-1967-0>.
- Muttucumaru N, Powers SJ, Elmore JS, Mottram DS, Halford NG. Effects of water availability on free amino acids, sugars, and acrylamide-forming potential in potato. *J Agric Food Chem*. 2015;63:2566–75.
- Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, Marton MJ. Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol Cell Biol*. 2001. <https://doi.org/10.1128/mcb.21.13.4347-4368.2001>.
- Nerlov C. The C/EBP family of transcription factors: a paradigm for interaction between gene expression and proliferation control. *Trends Cell Biol*. 2007. <https://doi.org/10.1016/j.tcb.2007.07.004>.
- Noree C, Monfort E, Shotelersuk V. Human asparagine synthetase associates with the mitotic spindle. *Biol Open*. 2018. <https://doi.org/10.1242/bio.038307>.
- Olea F, Pérez-García A, Cantón FR, Rivera ME, Cañas R, Ávila C, Cazorla FM, Cánovas FM, de Vicente A. Up-regulation and localization of asparagine synthetase in tomato leaves infected by the bacterial pathogen *Pseudomonas syringae*. *Plant Cell Physiol*. 2004. <https://doi.org/10.1093/pcp/pch092>.
- Osuna D, Gálvez-Valdivieso G, Piedras P, Pineda M, Aguilar M. Cloning, characterization and mRNA expression analysis of *PVAs1*, a type I asparagine synthetase gene from *Phaseolus vulgaris*. *Planta*. 2001. <https://doi.org/10.1007/s004250000513>.
- Perochon A, Váry Z, Malla KB, Halford NG, Paul MJ, Doohan FM. The wheat SnRK1a family and its contribution to Fusarium toxin tolerance. *Plant Sci*. 2019. <https://doi.org/10.1016/j.plantsci.2019.110217>.
- Qu C, Hao B, Xu X, Wang Y, Yang C, Xu Z, Liu G. Functional research on three presumed asparagine Synthetase family members in poplar. *Genes*. 2019;10:326.
- Raffan S, Halford NG. Acrylamide in food: progress in and prospects for genetic and agronomic solutions. *Ann Appl Biol*. 2019. <https://doi.org/10.1111/aab.12536>.
- Raffan S, Oddy J, Halford NG. The sulphur response in wheat grain and its implications for acrylamide formation and food safety. *Int J Mol Sci*. 2020;21:3876.
- Ramírez-González RH, Borrill P, Lang D, et al. The transcriptional landscape of polyploid wheat. *Science*. 2018. <https://doi.org/10.1126/science.aar6089>.
- Rapp M, Schwadorf K, Leiser WL, Würschum T, Longin CFH. Assessing the variation and genetic architecture of asparagine content in wheat: what can plant breeding contribute to a reduction in the acrylamide precursor? *Theor Appl Genet*. 2018. <https://doi.org/10.1007/s00122-018-3163-x>.
- Rashmi D, Barvkar VT, Nadaf A, Mundhe S, Kadoo NY. Integrative omics analysis in *Pandanus odorifer* (Forssk.) Kuntze reveals the role of asparagine synthetase in salinity tolerance. *Sci Rep*. 2019. <https://doi.org/10.1038/s41598-018-37039-y>.
- Rodriguez M, Parola R, Andreola S, Pereyra C, Martínez-Noël G. TOR and SnRK1 signaling pathways in plant response to abiotic stresses: do they always act according to the “yin-yang” model? *Plant Sci*. 2019. <https://doi.org/10.1016/j.plantsci.2019.110220>.
- Ruberti C, Brandizzi F. Conserved and plant-unique strategies for overcoming endoplasmic reticulum stress. *Front Plant Sci*. 2014. <https://doi.org/10.3389/fpls.2014.00069>.
- Saia S, Ruisi P, Fileccia V, di Miceli G, Amato G, Martinelli F. Metabolomics suggests that soil inoculation with arbuscular mycorrhizal fungi decreased free amino acid content in roots of durum wheat grown under N-limited, P-rich field conditions. *PLoS ONE*. 2015. <https://doi.org/10.1371/journal.pone.0129591>.
- Salvioli A, Zouari I, Chalot M, Bonfante P. The arbuscular mycorrhizal status has an impact on the transcriptome profile and amino acid composition of tomato fruit. *BMC Plant Biol*. 2012. <https://doi.org/10.1186/1471-2229-12-44>.
- Seiffert B, Zhou Z, Wallbraun M, Lohaus G, Möllers C. Expression of a bacterial asparagine synthetase gene in oilseed rape (*Brassica napus*) and its effect on traits related to nitrogen efficiency. *Physiol Plant*. 2004. <https://doi.org/10.1111/j.1399-3054.2004.00361.x>.
- Shi L, Wu Y, Sheen J. TOR signaling in plants: conservation and innovation. *Development*. 2018. <https://doi.org/10.1242/dev.160887>.
- Sieciechowicz KA, Ireland RJ. Isolation and properties of an asparaginase from leaves of *Pisum sativum*. *Phytochemistry*. 1989. [https://doi.org/10.1016/S0031-9422\(00\)97967-6](https://doi.org/10.1016/S0031-9422(00)97967-6).
- Sieciechowicz KA, Joy KW, Ireland RJ. The metabolism of asparagine in plants. *Phytochemistry*. 1988a. [https://doi.org/10.1016/0031-9422\(88\)84071-8](https://doi.org/10.1016/0031-9422(88)84071-8).
- Sieciechowicz KA, Ireland RJ, Joy KW. Diurnal changes in asparaginase activity in pea leaves: II. Regulation of activity. *J Exp Bot*. 1988b. <https://doi.org/10.1093/jxb/39.6.707>.
- Sieciechowicz KA, Joy KW, Ireland RJ. Diurnal changes in asparaginase activity in pea leaves: I. The requirement for light for increased activity. *J Exp Bot*. 1988c. <https://doi.org/10.1093/jxb/39.6.695>.
- Sikalidis AK, Stipanuk MH. Growing rats respond to a sulfur amino acid-deficient diet by phosphorylation of the α subunit of eukaryotic initiation factor 2 heterotrimeric complex and induction of adaptive components of the integrated stress response. *J Nutr*. 2010. <https://doi.org/10.3945/jn.109.120428>.
- Siu F, Bain PJ, Leblanc-Chaffin R, Chen H, Kilberg MS. ATF4 is a mediator of the nutrient-sensing response pathway that activates the human asparagine synthetase gene. *J Biol Chem*. 2002. <https://doi.org/10.1074/jbc.M201959200>.
- Soares E, Shumbe L, Dauchot N, Notte C, Prouin C, Maudoux O, Vanderschuren H. Asparagine accumulation in chicory storage roots is controlled by translocation and feedback regulation of asparagine biosynthesis in leaves. *bioRxiv*. 2020. <https://doi.org/10.1101/2020.04.27.063412>.
- Sodek L, Lea PJ, Mifflin BJ. Distribution and properties of a potassium-dependent asparaginase isolated from developing seeds of *Pisum sativum* and other plants. *Plant Physiol*. 1980. <https://doi.org/10.1104/pp.65.1.22>.
- Sormani R, Delannoy E, Lageix S, Bitton F, Lanet E, Saez-Vasquez J, Deragon JM, Renou JP, Robaglia C. Sublethal cadmium intoxication in *Arabidopsis thaliana* impacts translation at multiple levels. *Plant Cell Physiol*. 2011. <https://doi.org/10.1093/pcp/pcr001>.
- Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert MC, Riediker S. Food chemistry: acrylamide from Maillard reaction products. *Nature*. 2002. <https://doi.org/10.1038/419449a>.
- Stewart GR, Larher F. Accumulation of amino acids and related compounds in relation to environmental stress. In: Mifflin BJ, editor. *The biochemistry of plants, a comprehensive treatise*, Volume 5, Amino acids and derivatives. Amsterdam: Elsevier; 1980. p. 609–35.
- Stockmann F, Weber EA, Schreiter P, Merkt N, Claupein W, Graeff-Hönninger S. Impact of nitrogen and sulfur supply on the potential of acrylamide formation in organically and conventionally grown winter wheat. *Agronomy*. 2018. <https://doi.org/10.3390/agronomy8120284>.
- Stockmann F, Weber EA, Mast B, Schreiter P, Merkt N, Claupein W, Graeff-Hönninger S. Acrylamide-formation potential of cereals: what role does

- the agronomic management system play? *Agronomy*. 2019. <https://doi.org/10.3390/agronomy9100584>.
- Su N, Kilberg MS. C/EBP homology protein (CHOP) interacts with activating transcription factor 4 (ATF4) and negatively regulates the stress-dependent induction of the asparagine synthetase gene. *J Biol Chem*. 2008. <https://doi.org/10.1074/jbc.M806874200>.
- Swann OG, Kilpatrick M, Breslin M, Oddy WH. Dietary fiber and its associations with depression and inflammation. *Nutr Rev*. 2019. <https://doi.org/10.1093/nutrit/nuz072>.
- Szabados L, Savouré A. Proline: a multifunctional amino acid. *Trends Plant Sci*. 2010. <https://doi.org/10.1016/j.tplants.2009.11.009>.
- Taeymans D, Wood J, Ashby P, et al. A review of acrylamide: an industry perspective on research, analysis, formation, and control. *Crit Rev Food Sci*. 2004. <https://doi.org/10.1080/10408690490478082>.
- Thiaville MM, Dudenhausen EE, Zhong C, Pan YX, Kilberg MS. Deprivation of protein or amino acid induces C/EBP β synthesis and binding to amino acid response elements, but its action is not an absolute requirement for enhanced transcription. *Biochem J*. 2008. <https://doi.org/10.1042/BJ20071252>.
- Tonin GS, Sodek L. Asparaginase, allantoinase and glutamine synthetase activities in soybean cotyledons grown in vitro. *Phytochemistry*. 1990. [https://doi.org/10.1016/0031-9422\(90\)87085-9](https://doi.org/10.1016/0031-9422(90)87085-9).
- USDA-APHIS. JR Simplot company petition (14-093-01p) for determination of nonregulated status for InnateTM potatoes with late blight resistance, low acrylamide potential, reduced black spot and lowered reducing sugars: Russet Burbank event W8; 2014.
- Wang HB, Liu DC, Sun JZ, Zhang AM. Asparagine synthetase gene *TaASN1* from wheat is up-regulated by salt stress, osmotic stress and ABA. *J Plant Physiol*. 2005;162:81–9.
- Weber EA, Graeff S, Koller WD, Hermann W, Merkt N, Claupein W. Impact of nitrogen amount and timing on the potential of acrylamide formation in winter wheat (*Triticum aestivum* L.). *Field Crop Res*. 2008. <https://doi.org/10.1016/j.fcr.2007.10.011>.
- Wek RC, Jiang HY, Anthony TG. Coping with stress: EIF2 kinases and translational control. *Biochem Soc Trans*. 2006. <https://doi.org/10.1042/BST0340007>.
- Whiteside MD, Garcia MO, Treseder KK. Amino acid uptake in arbuscular mycorrhizal plants. *PLoS ONE*. 2012. <https://doi.org/10.1371/journal.pone.0047643>.
- Wurzinger B, Nukarinen E, Nägele T, Weckwerth W, Teige M. The snrk1 kinase as central mediator of energy signaling between different organelles. *Plant Physiol*. 2018. <https://doi.org/10.1104/pp.17.01404>.
- Xu F, Oruna-Concha MJ, Elmore JS. The use of asparaginase to reduce acrylamide levels in cooked food. *Food Chem*. 2016. <https://doi.org/10.1016/j.foodchem.2016.04.105>.
- Xu HW, Curtis TY, Powers SJ, Raffan S, Gao RH, Huang JH, Heiner M, Gilbert DR, Halford NG. Genomic, biochemical, and modelling analyses of asparagine synthetases from wheat. *Front Plant Sci*. 2018. <https://doi.org/10.3389/fpls.2017.02237>.
- Yadav AK, Carroll AJ, Estavillo GM, Rebetzke GJ, Pogson BJ. Wheat drought tolerance in the field is predicted by amino acid responses to glasshouse-imposed drought. *J Exp Bot*. 2019. <https://doi.org/10.1093/jxb/erz224>.
- Yang R, Wek SA, Wek RC. Glucose limitation induces GCN4 translation by activation of Gcn2 protein kinase. *Mol Cell Biol*. 2000. <https://doi.org/10.1128/mcb.20.8.2706-2717.2000>.
- Zhang Y, Dickinson JR, Paul MJ, Halford NG. Molecular cloning of an arabidopsis homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation. *Planta*. 2003. <https://doi.org/10.1007/s00425-003-1025-4>.
- Zhang Y, Andralojc PJ, Hey SJ, Primavesi LF, Specht M, Koehler J, Parry MAJ, Halford NG. Arabidopsis sucrose non-fermenting-1-related protein kinase-1 and calcium-dependent protein kinase phosphorylate conserved target sites in ABA response element binding proteins. *Ann Appl Biol*. 2008a. <https://doi.org/10.1111/j.1744-7348.2008.00302.x>.
- Zhang Y, Wang Y, Kanyuka K, Parry MAJ, Powers SJ, Halford NG. GCN2-dependent phosphorylation of eukaryotic translation initiation factor-2 α in Arabidopsis. *J Exp Bot*. 2008b. <https://doi.org/10.1093/jxb/ern169>.
- Zhang Y, Primavesi LF, Jhurrea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schlupepmann H, Delatte T, Wingler A, Paul MJ. Inhibition of SNF1-related protein kinase activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiol*. 2009. <https://doi.org/10.1104/pp.108.133934>.
- Zhang J, Fan J, Venneti S, et al. Asparagine plays a critical role in regulating cellular adaptation to glutamine depletion. *Mol Cell*. 2014. <https://doi.org/10.1016/j.molcel.2014.08.018>.
- Zhao FJ, Hawkesford MJ, McGrath SP. Sulphur assimilation and effects on yield and quality of wheat. *J Cereal Sci*. 1999. <https://doi.org/10.1006/jcrs.1998.0241>.
- Zheng T, Qi PF, Cao YL, et al. Mechanisms of wheat (*Triticum aestivum*) grain storage proteins in response to nitrogen application and its impacts on processing quality. *Sci Rep*. 2018. <https://doi.org/10.1038/s41598-018-30451-4>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

