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**Review Article**

# **New Innovations in Seed Morphology, and Plant Physiology for Crop Protection**

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**\*Corresponding author:** Muhammad Saleem Department of Botany, University of Agriculture Faisalabad **Abstract**

The seed is a mainly reproductive unit of the spermatophyta and links the subsequent generations. Other essential seed activities include survival in arid, cold, or other adverse conditions and dispersal. There is a lot of variety in the internal and external architecture of seeds. OsMKP1, a mitogen-activated protein kinase phosphatase, is encoded by GSN1. Rice glume cells proliferate when GSN1 expression is suppressed, producing larger but fewer rice grains. The GSN1 directly interacts with OsMAPK6 and dephosphorylates it, rendering it inactive. Thus, precise regulation of OsMAPK6 activity via reversible phosphorylation is essential for regulating the rice grain size. Some genes also negatively control the grain width and weight by inhibiting cell proliferation. The embryo, which is made up of cotyledons, hypocotyl, and radicle; the endosperm, which feeds the growing embryo; and the seed coat, which envelops the embryo and the endosperm, are the three main parts of plant seeds, each of which has unique biological functions and outcome. The growing seed can benefit from the ability to predict when a sugar burst would arrive through the phloem, since this would allow the seed to quickly adapt to its storage product synthesis. However, there are still certain obstacles that prevent the use against a variety of insect species. The adoption of various formulation procedures that improve dsRNA persistence and cellular absorption in insects is expected to overcome these issues, which are mostly related to the varying RNAi sensitivity of oral RNAi in insects. The CRISPR/Cas system has become the most popular being shown to be useful for editing the genome of plants, its uses in plants have grown significantly in comparison to other technologies.

**Keywords**: Protein kinase phosphatase, precise regulation, inhibiting cell proliferation.

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## **INTRODUCTION**

There are some varieties of the plants with novel genetic modifications. The seed is the reproductive unit of the spermatophyta (seed plants) and links the subsequent generations. Other essential seed activities include survival in arid, cold, or other adverse conditions and dispersal. There is a lot of variety in the internal and external architecture of seeds [1, 2]. The endosperm and embryo's size and placement, the seed's overall shape and dimensions, and its structure, texture, and color are some examples of these variances. They have a significant connection to germination and dispersion methods. The weight of an orchid seed can be as low. The legume seeds

may be the heaviest, weighing more, although the singleseeded fruit of the palm *Lodoicea maldivica* weighs. The three components of a seed are the embryo, endosperm and occasionally perisperm, and seed coat. The endosperm and embryo are produced via double fertilization, while the seed coat is created by the ovular, maternal tissues. The seed habit is a significant advancement in the evolution of higher plants. There are several evolutionary advantages to seed plants over spore-producing plants. While fertilization takes place inside the protective structures of the mother plant to the growing embryo receives sustenance from plant. In addition, the embryo is frequently given storage material and is shielded by the seed coat. Due to these features,

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the seed plants were so effective that took over the earth's terrestrial habitats [3, 4].

There are significant parallels between the evolution of viviparous mammals from egg-producing vertebrates and the emergence of the seed habit in spermatophytes, which occurred roughly during the same geological epoch. In addition to their significance for the plants themselves, seeds play a major role in both human and animal food chains [4, 5]. Seeds are likely the most valuable element of plants to humans. Wheat, oats, barley, rice, maize, and other crops are produced by the vast, cultivated grasses and cereals. Legumes like peanuts, beans, and soybeans have seeds that are high in either proteins or carbohydrates. Additionally, seeds are used as condiments, spices, and beverages such as coffee, cocoa, and strong drinks; they are also utilized as medications in the form of seed extracts; and they are used to make industrial and edible oils from linseed, peanuts, soybeans, coconuts, sunflowers, and other plants and some industrial uses of seeds. One unique benefit of seeds is their ability to store food [6, 7].

#### **Molecular Mechanism in the Seed Formation**

OsMKP1, a mitogen-activated protein kinase phosphatase, is encoded by GSN1. Rice glume cells proliferate when GSN1 expression is suppressed, producing larger but fewer rice grains. In contrast to OsMKK4, GSN1 directly interacts with OsMAPK6 and dephosphorylates it, rendering it inactive. Thus, precise regulation of OsMAPK6 activity via reversible phosphorylation is essential for regulating rice grain size [8, 9]. OsbZIP47 negatively controls grain width and weight by inhibiting cell proliferation, whereas WG1

encodes a CC glutaredoxin that stimulates grain growth by increasing cell proliferation. Through the recruitment of the transcriptional co-repressor ASP1, WG1 interacts with the transcription factor OsbZIP47 and inhibits its transcriptional activity. Furthermore, E3 ubiquitin ligase GW2 ubiquitinates WG1 and then degrades it. According to genetic study, the three proteins coordinate grain size and weight by forming a molecular regulatory module called GW2-WG1-OsbZIP47[8]. The cultivated peas have been used to study the development of legume seeds. A temporary storage organ in the early stages of development, the legume SC stores proteins and carbohydrates before the embryo begins to store them. Embryo growth is hindered when this SC storage activity is changed [9, 10].

The SC is metabolically very active during seed filling and facilitates the production of storage compounds in the filial tissues by transferring organic resources from the phloem. The SC cells experience senescence during seed growth and desiccation. The developed SC serves as the confined embryo's barrier of defense [10, 11]. In addition to providing mechanical protection from the environment, it also acts as a mediator for environmental information, particularly when it comes to the breaking of dormancy and subsequent seed germination. In these procedures, the SC's content and structure are crucial. Secondary cell walls that are loaded with fats, waxes, and polyphenolic compounds provide mechanical strength and act as insect predator deterrents. It has recently been demonstrated that the SC includes active enzymes that are released upon hydration and can last for decades [10, 11].



**Fig-1: The anatomy and approaches in the seed and molecular development**

The hilum, and micropyle are some of the physically unique areas that make up the SC. At seed maturity, the detached funiculus forms a scar known as the hilum [11, 12]. During seed germination, the radicle

emerges through the micropyle, which is created at the edges of the integuments. On the other side of the hilum from the micropyle lies a slightly recessed region known as the raphe. The architecture and mechanisms governing

water entrance into legume seeds are still being debated. It was believed that the micropyle or hilum had a significant role in regulating water intake. Similar to this, certain legumes have the ability to regulate the release of seed dormancy, whereas other species like soybean and pea do not. The tracheid bar and hilum contribute to seed desiccation, although they are not a major source of water input for peas during imbibition. Unlike peas, the hilum's role as a hygroscopically triggered valve has been in some legume species [9-12].

Cell expansion is a mechanical process that is aided by water uptake-driven wall extension, stress relaxation, and cell wall loosening. Cellulases, enzymes that change pectin and xyloglucan, and apoplastic i.e., found in the plant cell wall rather than the protoplast reactive oxygen species (aROS) are important participants in this process [13, 14]. The internal turgor pressure drops when the cell wall's load-bearing polymers relax. This causes the cell to absorb more water until the turgor pressure and the cell wall's constraint are balanced once more. According to a contemporary concept of the plant cell wall, wall extensibility is caused by biomechanical hotspots illustrated a summary of the structure and function of the seed and fruit coat. The palisade layer of lignified macrosclerids saturated with water-repellent phenolic and suberin-like compounds is responsible for the water-impermeability of many legume seed coverings. In *M. truncatula* and soybean, genes governing PY, the formation of the cuticle, and the seed coat palisade layer have been found. When PY is broken, water-gap structures that serve as environmental signal detectors, like the lens in legume seeds, open [14, 15].

Many RNA processing mutations for electron chain subunits exhibit decreased ABA sensitivity, mitochondria are linked to ABA sensitivity and play a crucial role in energy delivery. The transcription control of the ABA biosynthesis gene, NCED, is regulated by retrograde, anterograde, and inter-organelle signals [16, 17]. ABA treatment had a minor impact on the mitochondrial dynamics linked to the germination state, supporting the idea that temperature and hydration are the sole physical factors that affect mitochondrial reactivation. The decrease in ABA sensitivity is linked to the best possible mitochondrion differentiation and function. Therefore, it is reasonable to assume that mitochondrial activity is impaired in dormant seeds [17, 18]. Nevertheless, it has long been known that cyanide and other oxidative phosphorylation inhibitors can end dormancy. There is still no explanation for this conundrum. The most likely explanation put out is the activation of the pentose phosphate pathway, which is the metabolic system that provides cells with decreasing energy. However, in both lab-grown and soil-grown seed bank experiences, GA biosynthesis gene involvement has also been documented in response to environmental signals in Arabidopsis seeds. The significance of pentose phosphate was further highlighted by the examination of

the whole transcriptome change caused by nitrate treatment during seed imbibition, which revealed the upregulation of genes related to nitrate assimilation and transport, hormone metabolism, and energy, including the glucose-6-phosphate dehydrogenase. It's interesting to note that many environmental cues, like light, nitrate, stratification, or after-ripening, caused dormancy release-related alterations at the gene expression level. Genes involved in the reserve mobilization, cell wall modification, and translation machinery are involved. These reversible variations in transcript abundance enable the dormancy cycling phenomenon, which happens when adverse environmental circumstances persist after the original hibernation relief, leading to the onset of a secondary dormancy. As seeds are exposed to a variety of temperature, light, nitrate, and moisture conditions as well as microbial environments in the soil, dormancy is really strictly controlled in natural settings [16-18].

The embryo, which is made up of cotyledon(s), hypocotyl, and radicle; the endosperm, which feeds the growing embryo; and the seed coat, which envelops the embryo and the endosperm, are the three main parts of plant seeds, each of which has unique biological functions and outcomes [19, 20]. In addition to acting as a mechanical barrier, it may also have a role in controlling germination and seed distribution. But in mature rapeseed seeds, the embryo is firmly encased in the seed coat while the endosperm degenerates. The appearance, structure, or development of oil and protein bodies in the rapeseed seed embryo has been documented in several research. Furthermore, based on the structural structure of the seeds, various investigations reported on the pigment dispersion in rapeseed seed coatings. There is a strong correlation between the plants and *B. napus* decreased oil content. Nevertheless, the seed structure and properties of ultrahigh of the oil content rapeseed [18, 19].

The chloroplasts then generate enough ATP and NAPDH in response to light exposure to satisfy local energy needs. Since both ATP and NADPH have extremely useful and it is expected that hardly much long-distance transport takes place from their manufacturing site [21, 22]. Like other nonphotosynthetic tissues, the pericarp's non-photosynthetic plastids are totally dependent on an external source of ATP. It is implied that seed photosynthesis does not directly contribute energy to assimilate storage in the endosperm due to spatial separation between the endosperm and the photosynthetically active pericarp. The seed coat's ability to intercept and process light provides the seed with the ability to sense its surroundings, which is combined with the hormonal, metabolic, and other signals that are delivered to the seed through the phloem. This is one plausible explanation for the evolutionary significance of the seed coat's ability to retain photosynthetic capacity. Sugar is exported into the phloem as assimilate produced in the leaf. The growing seed can benefit from the ability to predict when a sugar burst would arrive through the phloem, since this would allow the seed to quickly adapt to its storage product synthesis. The maintenance of strong circadian rhythms are significantly impacted by the photosynthesis. In this sense, maintaining seed photosynthesis can help adjust the metabolism of the seed to the amount and kind of light that the mother plant has access to [22, 23].

#### **Emerging Applications**

The effectiveness and efficiency of crop protection strategies might vary from one growing season to the next, just as environmental and climatic variables [24, 25]. Second, a variety of context-specific elements, including the production, environmental, or institutional context, as well as the indicators employed, influence the journey from the adoption of measures to their actual impact on pesticide risk reduction the ultimate policy target. There will be a more or less direct correlation between pesticide risk reduction and several indices. Third, because pest control techniques frequently depend on one another, combining them may result in non-linear changes in efficacy and efficiency. As a result, the uptake of various bundles of measures

may be evaluated differently from the uptake of individual measures or other practice combinations. Furthermore, there is a dearth of timely and reliable data on pesticide usage, which leaves us frequently in the dark about what farmers are doing, how, and where. These integrated techniques share the objective of ensuring high food production productivity and efficient pest control, minimizing negative effects on the environment and human health, and ensuring the economic sustainability of agriculture [25, 26].

Various methods could permit the use of synthetic chemical pesticides either completely or in small amounts e.g. integrated pest management, agroecological crop protection. A combination of strategies, including preventive, biological control, agronomic solutions like field cleanliness and crop rotations, technical solutions like mechanical weed control and smart farming, and the use of resistant and adaptable cultivars, can be used to accomplish this. Due to variations in adoption criteria, costs, rewards, and dangers, alternative techniques may also have comparable effects on farmer adoption [24-26].



**Fig-2: The molecular approaches for genetic transfer for new varieties**

Sharing information about new innovations and best practices is especially crucial because crop security is a complicated and ever-evolving topic. Because it was the empirical emphasis of the is derived, it concentrates on the dissemination of knowledge regarding crop protection techniques. Focusing on crop protection measures allows us to restrict our research to communication with a comparable sort of information, even if many of the applicable to transmission of other forms of knowledge [26, 27]. Furthermore, crop health is essential to crop production in order to maintain the yield's quality and quantity.

Nowadays, the idea of integrated pest management, or IPM, incorporates other agronomic techniques, and the use of pesticides is the last resort.

However, many farmers still use pesticides to protect their crops. Recommendations for the optimal use of crop protection information in agricultural methods have not been compiled. The process of putting research knowledge into practice is examined in further detail with a focus on the particular knowledge-to-action (KTA) gap that may from being used effectively [25, 26].

Host-induced gene silencing (HIGS), a technique that involves producing dsRNAs in plants to quiet key genes, has proved effective in increasing resistance to pests or diseases for a number of decades. HIGS technology is regarded as the best crop breeding technique because of its efficacy, environmental friendliness, and independence from resistant cultivars. However, many crop species lack transformation technology, which restricts the wider use of HIGS. An age of RNAi-based biopesticides was crushed in by the alternative technique of spray-induced gene silencing (SIGS), which avoids crop modification [27, 28]. Only a small number of these sRNAs significantly reduced TF expression. The effectiveness of exogenous dsRNA treatment against tomato spotted wilt virus in plants varies depending on the area that is targeted. The natural cleavage hotspots in the 3' untranslated region (UTR) of the cucumber mosaic virus (CMV) genome revealed in order to design efficient RNA silencing and viral resistance in plants. High resistance to CMV is conferred by artificial microRNAs that target potential sRNA accessible target sites. sRNAs' positional influence on mRNAs using a reliable experimental methodology [29,30]. All things considered, dsRNAs' ability to target distinct places on the same RNA varies greatly. dsRNAs and dsRNAs isolated from virus-infected plants elicited the usual PTI responses, where dsRNAs act as conserved molecular patterns linked to microbes or pathogens. SERK1 and a certain dsRNA receptor, but not DCLs, were involved in the signalling cascade. There was no observable reactive oxygen species burst, in contrast to the bacterial and fungal elicitor-mediated PTI. Remarkably, viral movement proteins from several viruses inhibited the dsRNA-induced host response, whereas dsRNA-induced PTI limited the development of virus infection by inducing callose deposition at plasmodesmata. More research is required to determine if dsRNA-triggered plant immunity influences how well biopesticides work. Exogenous dsRNAs also use transfection reagents and high RNA concentrations to boost the innate immune response in animal models in vitro. The dsRNA-mediated innate immune response involves RNA helicases, dsRNA binding protein kinases, and toll-like receptors [29, 30].

Because the unprocessed dsRNAs are found in xylem vessels and the apoplastic space, high-pressure spraying and petiole adsorption were successful in preventing Fusarium crown and root rot without causing plant processing. Fungal DICER proteins will eventually break the dsRNAs into siRNA after the fungi have ingested them intact, improving the ability of systemic RNA interference to target fungal genes [31, 32]. When RNAi effects are elicited against insects to enable apoplastic RNA transport. RNAi must take place within the plant cell and permit symplastic RNA transport, which may be accomplished by high-pressure spraying, in order to produce RNAi effects against viruses and endogenous plant genes, in contrast to fungus and insects [33, 34].

The necessity of risk evaluations and dsRNAbased product management is a significant component of topical RNAi use [32-34]. It outline a number of important factors to take into account when conducting risk assessments. Since dsRNAs can be used as an active component in biopesticides, which pose dangers akin to those of conventional pesticides, the risks associated

with topical RNAi are distinct from those associated with conventional genetic alteration. If these nanotechnologies are to be commercialized, risk assessment and management factors that must be taken into account include possible environmental pollution, skin and respiratory irritation or injury, and more. However, possible off-target silencing effects on target and non-target species that might be triggered with sufficient sequence similarity between dsRNA and offtarget transcripts are the specific issues associated with RNAi silencing activities. As the technology enters the market, it is crucial to address the worry about potential off-target consequences to guarantee public support. To reduce off-target hits, RNAi target sequence designs should be extremely specific, homologous, and have very little sequence similarity to off-target transcripts [34, 35].

The functional RNA transfer between interacting species depicted the importance of RNA trafficking in host and microbe interactions. Fungal sRNAs enter host cells and Arabidopsis AGO1 to silence plant immunity genes, weakening host immunity to promote fungal infection. This phenomenon is known as cross-kingdom RNA interference (RNAi) and has been observed in the plant between the fungal pathogen Botrytis cinerea and the model plant Arabidopsis thaliana [35, 36]. DICER in most animals and DICERlike (DCL) in plants and fungi are ribonuclease III enzymes that convert endogenously manufactured or exogenously supplied dsRNAs into 20-30 nucleotide long duplexes. Following that, the sRNA duplexes are loaded into certain cytoplasmic members of the AGO protein family. After then, the RNA duplex unwinds, the passenger RNA strand is released, and other proteins are recruited to form an RNA-induced silencing complex (RISC). After the RISC is imported into the nucleus, the retained guide RNA strand guides the RISC complex to either a DNA target or a complementary RNA target in the cytoplasm [36, 37].

The length and structure of the dsRNA as well as the delivery method have an impact on its uptake illustrate a number of dsRNA chemical changes and delivery methods. The dsRNA must be released from the endosome after entering the cell by endocytosis in order to interact with the RNAi machinery (dcr-2 and RISC) and cause the targeted gene to be knocked down. After the endosome is acidified, endosomal release takes place. Low sensitivity to RNAi in Lepidoptera is caused by the absence of endosomal release of the dsRNA, according to research done with the autumn armyworm, Spodoptera frugiperda. The snf7 dsRNA provides yet another illustration of the possible of absorption in RNAi efficiency. Cross-resistance to other dsRNAs seen in resistance to snf7 dsRNA, and microscopy investigations revealed a connection between resistance and dsRNA uptake [38-40].

### **CONCLUSION AND FUTURE PERSPECTIVES**

The drastic changes in crop protection are now required rather than the minor tweaks made to IPM, which favoured intensive agriculture, which relies heavily on monoculture and copious amounts of inputs, especially pesticides. The RNAi-based products, which target insects that are extremely sensitive to dietary absorption of dsRNA. However, there are still certain obstacles that prevent the use against a variety of insect species. The adoption of various formulation procedures that improve dsRNA persistence and cellular absorption in insects is expected to overcome these issues, which are mostly related to the varying RNAi sensitivity of oral RNAi in insects.

CRISPR/Cas systems have emerged as the most widely used GE technology in recent years. Because the specificity of editing is determined by the nucleotide complementarity of the guide RNA to a particular region without the need for intricate protein fabrication, the CRISPR/Cas systems are more effective and simple for genome editing than other SDNs. As a result, CRISPR/Cas techniques can be used for functional study of genes. When used in agricultural development fields, GE may drastically save labor costs and other expenses while also greatly speeding up the insertion of desirable features.

Notably, the CRISPR/Cas system has become the most popular and innovative SSN. Despite just being shown to be useful for editing the genome of plants, its uses in plants have grown significantly in comparison to other NPBTs. In many economically significant crops, including rice potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), soybean (*Glycine max*), and brassicas, research employing CRISPR has introduced significant agricultural traits, such as resistance to heat, cold, and herbicides; and increased grain size and weight. Crucially, it used transgene-free techniques to achieve such genomic modifications.

The development of climate-smart crops, sometimes referred to as advanced breeding technologies, could employ CRISPR-Cas technologies to create both transgenic and non-transgenic agricultural varieties. Non-transgenic variations can be produced by transferring an allele from one variety of the same species to another or by creating null alleles by templatefree genome editing. This may be accomplished, for example, by transferring a drought-resistant gene from a wild type to a commercial line. Their products can be promptly introduced because the kinds created in this way are comparable to those created using traditional breeding methods. Transgenic crops are produced by inserting transgenes into predetermined locations using a template. Understanding CRISPR-Cas genome-edited crops beforehand is essential to their development.

Compared to traditional breeding, new breeding methods allow scientists to swiftly and precisely introduce the required features. Genome editing with

CRISPR/Cas9 is a fundamentally innovative method. Future research will focus heavily on the use of genome editing techniques to improve crops' production, nutritional value, disease resistance, and other characteristics. It has been widely used in various plant systems during the past years for functional research, biotic and abiotic stress management, and the enhancement of other significant agronomic features. The majority of the gap is preliminary and requires improvement, even though a number of changes to this technology must increase on-target efficiency. However, genome editing using CRISPR/Cas9 will become more common and a necessary method to get appropriately.

Although several studies appear promising, before these RNAi products can rival the conventional chemical pesticides now in use for controlling a variety of insect species.

#### **REFERENCES**

- 1. Development of angiosperm seeds. In: Kozlowski TT (ed) Seed biology, vol 1. Academic Press, New York London, pp 77.
- 2. Boesewinkel, F. D. (1978). Development of ovule and testa in Rutaceae III. Some representatives of the Aurantioideae. *Acta Bot Neerl*, 27, 341-354.
- 3. Guérin, P. (1899). Recherches sur le développement du tégument séminal et du péricarpe des Graminées. *Ann Sci Nat Bot, 9*(8), 1-59.
- 4. Guignard, L. (1893). Note sur l'origine et la structure du tégument séminal chez les Capparidées, Résédacées, Hypericacées, Balsaminées et Linacées. *Bull Soc Bot Fr*, 40, 56–59.
- 5. Olsen, S., & Olsen, J. D. (1980). The seed ofBoschniaka hookeri (Orobanchaceae). *Bot Tidsskr*, 75, 159-172.
- 6. Pate, J. S., & Gunning, B. E. S. (1972). Transfer cells. *Annu Rev Plant Physiol*, 23, 173–196.
- 7. Periasamy, K. (1962). The ruminate endosperm: Development and types of rumination. In: Plant embryology: A symposium. CSIR, New Delhi, pp  $62 - 74.$
- 8. Guo, T., Chen, K., Dong, N. Q., Shi, C. L., Ye, W. W., Gao, J. P., ... & Lin, H. X. (2018). GRAIN SIZE AND NUMBER1 negatively regulates the OsMKKK10-OsMKK4-OsMPK6 cascade to coordinate the trade-off between grain number per panicle and grain size in rice. *The Plant Cell*, *30*(4), 871-888.
- 9. Xu, R., Duan, P., Yu, H., Zhou, Z., Zhang, B., Wang, R., ... & Li, Y. (2018). Control of grain size and weight by the OsMKKK10-OsMKK4-OsMAPK6 signaling pathway in rice. *Molecular Plant*, *11*(6), 860-873.
- 10. Marbach, I., & Mayer, A. M. (1975). Changes in catechol oxidase and permeability to water in seed coats of Pisum elatius during seed development and maturation. *Plant Physiol*, 56, 93–96. doi: 10.1104/pp.56.1.93
- 11. Reeve, R. M. (1946). Ontogeny of the sclereids in the integument of Pisum sativum L. *Am J Bot*, 33, 806–816.
- 12. Spurný, M. (1954). Cell wall structure of the epidermal cells of the pea seed coat Pisum sativum L. *Preslia*, 26, 79-88.
- 13. Sperber, K., Steinbrecher, T., Graeber, K., Scherer, G., Clausing, S., Wiegand, N., ... & Mummenhoff, K. (2017). Fruit fracture biomechanics and the release of Lepidium didymum pericarp-imposed mechanical dormancy by fungi. *Nature Communications*, *8*(1), 1868.
- 14. Munz, E., Rolletschek, H., Oeltze‐Jafra, S., Fuchs, J., Guendel, A., Neuberger, T., ... & Borisjuk, L. (2017). A functional imaging study of germinating oilseed rape seed. *New Phytologist*, *216*(4), 1181- 1190.
- 15. Yan, D., Easwaran, V., Chau, V., Okamoto, M., Ierullo, M., Kimura, M., ... & Nambara, E. (2016). NIN-like protein 8 is a master regulator of nitratepromoted seed germination in Arabidopsis. *Nature communications*, *7*(1), 13179.
- 16. Delgado-Sánchez, P., Ortega-Amaro, M. A., Jiménez-Bremont, J. F., & Flores, J. (2011). Are Fungi Important for Breaking Seed Dormancy in Desert Species? Experimental Evidence in Opuntia streptacantha (Cactaceae): Fungi Break Seed Dormancy in Opuntia. *Plant Biol*, 13, 154–159.
- 17. Siloto, R. M. P., Findlay, K., Lopez-Villalobos, A., Yeung, E. C., & Nykiforuk, C. L. (2006). The accumulation of oleosins determines the size of seed oil bodies. *Plant Cell*, 18, 1961-1974.
- 18. Tzen, J. T. C., Cao, Y. Z., Laurent, P., Ratnayake, C., & Huang, A. H. C. (1993). Lipids, proteins, and structure of seed oilbodies from diverse species. *Plant Physiol*, 101, 267–276.
- 19. Mantese, A. I., Medan, D., & Hall, A J. (2006). Achene structure, development and lipid accumulation in sunflower cultivars deffering in oil content at maturity. *Ann Bot*, 97, 999–1010.
- 20. Bukhari, S. S., & Parkes, R. S. (2023). Assessing the impact of draught load pulling on welfare in equids. *Frontiers in Veterinary Science*, 10, 1214015.
- 21. Bukhari, S. S., & Parkes, R. S. (2023). Assessing the impact of draught load pulling on welfare in equids. *Frontiers in Veterinary Science*, 10, 1214015.
- 22. Bukhari, S. S., McElligott, A. G., & Parkes, R. S. (2021). Quantifying the impact of mounted load carrying on equids: a review. *Animals, 11*(5), 1333.
- 23. Bukhari, S. S., Parkes, R. S., Sneddon, L. U., & McElligott, A. G. (2024). The behavior and welfare of neglected species: some examples from fish and mammals. *Peer J*, 12, e17149.
- 24. Kapil, R. N., & Tiwari, S. C. (1978). The integumentary tapetum. *Bot Rev*, 44, 457–490.
- 25. Bradshaw, N. J., Parham, C. J., Croxford, A. C., & Council Brit Crop Protect. (1996). The Awareness, Use and Promotion of Integrated Control Techniques of Pests, Diseases & Weeds in British Agriculture and Horticulture. Brighton Crop

Protection Conference: Pests & Diseases – 1996, 1- 3.

- 26. Brinks, H., & de Kool, S. (2006). Farming with Future: Implementation of Sustainable Agriculture Through a Network of Stakeholders. Edited by H. Langeveld and N. Roling. Changing European Farming Systems for a Better Future: New Visions for Rural Areas.
- 27. Zhao, Q., Zhao, Z., Fan, X., Yuan, Z., Mao, Q., & Yao, Y. (2021). Review of machine learning methods for RNA secondary structure prediction. *PLoS computational biology*, *17*(8), e1009291.
- 28. Zhu, K. Y., & Palli, S. R. (2020). Mechanisms, applications, and challenges of insect RNA interference. *Annual review of entomology*, *65*(1), 293-311.
- 29. Townshend, R. J., Eismann, S., Watkins, A. M., Rangan, R., Karelina, M., Das, R., & Dror, R. O. (2021). Geometric deep learning of RNA structure. *Science*, *373*(6558), 1047-1051. https://doi.org/10.1126/science.abe5650.
- 30. Underwood, J. G., Uzilov, A. V., Katzman, S., Onodera, C. S., Mainzer, J. E., Mathews, D. H., ... & Haussler, D. (2010). FragSeq: transcriptome-wide RNA structure probing using high-throughput sequencing. *Nature methods*, *7*(12), 995-1001.
- 31. Uslu, V. V., Bassler, A., Krczal, G., & Wassenegger, M. (2020). High-pressure-sprayed double stranded RNA does not induce RNA interference of a reporter gene. *Frontiers in Plant Science*, *11*, 534391.
- 32. OECD. (2020). Considerations for the Environmental Risk Assessment of the Application of Sprayed or Externally Applied ds-RNA-Based Pesticides; OECD: Paris, France. Darsan Singh, J. K., Mat Jalaluddin, N. S., Sanan-Mishra, N., & Harikrishna, J. A. (2019). Genetic modification in Malaysia and India: current regulatory framework and the special case of non-transformative RNAi in agriculture. *Plant cell reports*, *38*, 1449-1463.
- 33. Romeis, J., & Widmer, F. (2020). Assessing the risks of topically applied dsRNA-based products to non-target arthropods. *Frontiers in plant science*, *11*, 679.
- 34. Casacuberta, J. M., Devos, Y., Du Jardin, P., Ramon, M., Vaucheret, H., & Nogué, F. (2015). Biotechnological uses of RNAi in plants: risk assessment considerations. *Trends in Biotechnology*, *33*(3), 145-147.
- 35. Kaksonen, M., & Roux, A. (2018). Mechanisms of clathrin-mediated endocytosis. *Nat Rev Mol Cell Biol*, 19, 313–326.
- 36. Knorr, E., Fishilevich, E., Tenbusch, L., Frey, M. L., Rangasamy, M., Billion, A., ... & Narva, K. E. (2018). Gene silencing in Tribolium castaneum as a tool for the targeted identification of candidate RNAi targets in crop pests. *Scientific reports*, *8*(1), 2061.
- 37. Kolliopoulou, A., Taning, C. N., Smagghe, G., & Swevers, L. (2017). Viral delivery of dsRNA for

control of insect agricultural pests and vectors of human disease: prospects and challenges. *Front Physiol*, 8, 399.

- 38. Mukherjee, K., Campos, H., & Kolaczkowski, B. (2013). Evolution of animal and plant dicers: early parallel duplications and recurrent adaptation of antiviral RNA binding in plants. *Mol Biol Evol*, 30, 627–641.
- 39. Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical pesticides and human health: the urgent need for a new concept in agriculture. *Front Public Health*, 4, 148.
- 40. Huvenne, H., & Smagghe, G. (2010). Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. *J Insect Physiol*, 56, 227– 235.