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Halo Priming of Seeds

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ABSTRACT

Seed priming is a commercially used technique for improving seed germination and vigour. It is a technique which involves uptake of water by the seed followed by drying to initiate the early events of germination upto the point of radicle emergence. The benefits include rapid, uniform and increased germination, improved seedling vigour and growth under a broad range of environments resulting in better stand establishment. The common feature in these priming is the controlled uptake of water. Also, the seeds used for priming elicit specific subcellular responses. This review article summarises the recent information available on the various physiological and subcellular processes associated with priming which lead to seed enhancement.

Key words: Seed priming, physiologial and biochemical changes, invigouration, improving germination, vigour

INTRODUCTION

Crop production depends heavily on planting of high quality seeds. Rapid and uniform emergence is almost important, it is the foundation on which stand establishment is based and potential yield is determined. Absolute longevity depends on initial seed quality, vigour and proper storage. In ancient days, various seed treatments were practiced as initial production techniques for improved productivity. One programmatic approach to increase crop production is seed invigouration (Farooq *et al.*, 2006). Seed invigouration strategies include hardening, osmohardening, osmoconditioning, hydropriming, hormonal priming, matri-priming and others (Windauer *et al.*, 2007). In the last two decades, seed priming is an effective seed invigouration method has become a common seed treatment to increase the rate and uniformity of emergence and crop establishments in most of the crops especially in advanced countries. Seed priming is a controlled hydration process that involves exposing the seeds to low water potentials that restrict germination, but permits pregerminative physiological and biochemical changes to occur (Khan, 1992). Upon rehydration, primed seeds may exhibit faster rates of germination, more uniform emergence, greater tolerance to environmental stress and reduced dormancy in many species (Khan, 1992).

Several literatures (Windauer *et al.*, 2007) revealed that seed priming could advance germination, improve the initial quality characters, improve field emergence, better establishment, crop stand and increase yields in many diverse environments. Generally, seed storage caused a decrease in the protein content which may be related to oxidation of the amino acids, due to the increase in the respiratory activity and advance in the deterioration process of the stored seeds.

Seed deterioration is associated with loss of membrane integrity, changes in enzymatic activities, decline in protein and nucleic acid synthesis and lesions in DNA (McDonald, 1999). Priming can reverse some of the aging-induced deteriorative events and thus improve seed performance (Taylor *et al.*, 1998). Priming is responsible to repair the age related cellular and subcellular damage of low vigor seeds that may accumulate during seed development (Bray, 1995). Priming of seed promotes germination by repair of the damaged proteins, RNA and DNA (Koehler *et al.*, 1997). Many seed priming treatments have been used to reduce the damage of aging and invigourate their performance in many crops (Farooq *et al.*, 2009).

However, a strong reduction in longevity is generally associated with primed seeds in many species, eventhough longevity is enhanced in some cases (Taylor *et al.*, 1998). The exact causes of faster deterioration of primed seeds remain unclear and still not established. Decreased longevity of primed seeds has been attributed to reduced DNA repair upon subsequent germination (Redfearn and Osborne, 1997). The promotion of germination with seed priming may take place for several reasons, but changes in metabolic levels are important during seed priming. It has been assumed that the onset of germination is associated with a rapid resumption of RNA and protein biosynthesis (Osborne, 1983). Limited numbers of investigations into the biochemical changes occurring in primed and subsequently germinated seeds have noted changes of pattern of RNA, protein and DNA synthesis (Dell'Aquila and Taranto, 1986). The goal of this article is to update the information on physilogical and biochemical basis of seed priming including recent references which have not been covered by earlier reviews.

Effect of seed priming on physiological seed quality parameters: In seed management, the success in application of any seed management technique depends upon the type of test, method of application, selection of crop, initial performance of the crop, selection of chemical, its concentration, duration of treatment and the purpose of implication. Seed priming is one of the pre-sowing seed management techniques where the seeds are partially soaked and subsequently dried back for invigorative effect that expresses on field emergence and extend up to yield. In rainfed areas, germination and subsequent seedling growth can be inhibited by adverse conditions in the field. Priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. Researchers had evaluated the influence of various priming treatments in different crops and recommended the suitable seed priming techniques both for field establishment and for improved productivity even under wider variations of growing atmosphere.

Cantliffe *et al.* (1984) primed lettuce seed (*Lactuca sativa* L. cv. Minetto) in aerated solutions of 1% K_3PO_4 and water at 15°C in the dark for various periods of time and reported that priming for 20 h in 1% K_3PO_4 improved the germination up to 86%. They also reported that cell division occurred at 21 h in water and at 27 h in 1% K_3PO_4 prior to radicle protrusion. Bradford (1986) revealed that seed priming has been successfully demonstrated to improve germination and emergence in many crops particularly seeds of vegetables and small seeded grasses. Furutani *et al.* (1986) expressed that onion seeds primed for 8 days at 10°C in -1.1 MPa of mannitol reduced the days to 50% germination by 46%. Odell and Cantliffe (1986) reported that tomato seeds primed with 1.5% K_3PO_4 +1% KNO₃ solution at 35°C had germinated more rapidly as compared to unprimed seeds. Basra *et al.* (1988) primed the maize seed in solutions of 2.5% K_2 HPO₄ and 2.5% K_2 HPO₄+KNO₃ found that germination accelerated at a chilling temperature and the effect of priming was largely retained after seeds had been dried back. Cantliffe (1991) revealed that lettuce seeds primed in one percent K_2PO_4 for 20 h in the dark with the addition of 100 ppm of

cytokinin reduced thermo dormancy and increased germination percentage. In summer squash Cavallaro *et al.* (1994) primed the seeds in aerated solution of $\text{KNO}_8+\text{K}_2\text{HPO}_4$ at 3% for 2 days revealed that priming improved the seedling emergence (97%) where as untreated seeds recorded 72% emergence. Anuradha *et al.* (1995) revealed that the hydration of freshly harvested cabbage seeds for two days at low temperature (10°C) had increased the emergence percent, speed of germination and days to 50% germination than the control.

Harris et al. (1999) revealed that on-farm seed priming (soaking seeds overnight in water) markedly improved the establishment and early vigor of maize resulting in faster development, earlier flowering, maturity and higher yields. McDonald (2000) reported that sunflower seeds primed with osmotic solution of PEG 800 improved the seedling length and dry mass of both shoot and root. Subbarao et al. (2001) reported that priming of black gram seeds with sodium molybdate (0.5 g L^{-1}) improved the seed yield of rice fallow situation in 19 trials conducted in 5 villages in Jharkhand and West Bengal. Maize seeds were subjected to hydropriming, osmotic priming (PEG-10,000) and matriconditioning with compost, pressmud and GA3. Results revealed that early germination was recorded in seeds matriconditioned with compost, pressmud and GA3 in laboratory and early emergence under field condition (Afzal et al., 2002). Kaur et al. (2003) observed that priming chickpea seeds with water caused early germination, increased seedling length, maximum biomass of root and shoot under salt stressed conditions when compared to non-primed seeds. Demer and Mavi (2004) haloprimed the watermelon seeds in KNO₃ 3% for 6 days at 20°C and found that priming decreased mean emergence time and increased seedling weight and hypocotyl length. Subedi and Ma (2005) evaluated the effect of seed priming with water, osmotic solution (2.5% KCl) and plant growth regulators (Indole acetic acid, cytokinin, ethephon and gibberelhc acid) on emergence, seedling vigor, nitrogen response and grain yield of corn. The results revealed that seed soaking with 20 ppm gibberellic acid (GA_{\circ}) solution for 30 min improved the seedling vigour.

Basra et al. (2003a) in wheat (Triticum aestivum cv. Auga) found that hydropriming for 24 h and hardening for 12 h (one cycle hydropriming, hardening and matriconditioning) were found better as expressed by germination and all other vigour parameters compared to matriconditioning with press mud for 24 h or halopriming with 100 mol m⁻³ CaCl₂ for 24 h. Farooq et al. (2006) reported in sunflower for seedling elongation H₂O₂, NaCl and GA3 were the best while for shoot and root dry weight, salicylic acid and H_2O_2 were promising. Mavi et al. (2006) found that improvement in plumule length was increased due to earlier germination induced by NaCl priming treatment. Sunflower seeds were hydro primed for 24 h, matriprimed for 24 and 48 h, osmoprimed with 0.5% KNO₃ for 12 h and 0.1% NaCl for 12 h. Hydropriming and osmopriming with NaCl resulted in early 50% emergence, increased plant population, achene yield and achene proteins, but plant height and achene oil contents were not affected significantly by different seed priming (Hussain et al., 2006). Kaya et al. (2006) reported that hydroprimed seeds of sunflower and wheat could germinate faster and produced longer seedling under salinity stress compared with untreated seeds. Ramzan et al. (2010) reported that gladiolus seeds primed with distilled water achieved 92% germination followed by 80% in KNO₃ 1 and 70% in KNO₃ 2%. It also reduced time required for 50% germination and it increased the seedling length of 14 cm. Mohan Kumar and Manonmani (2011) reported that sunflower hybrid seeds haloprimed with 2% KNO₈ for 6 h increase the germination speed, maximize the germination, vigour and field emergence (Table 1).

Effect of seed priming on biochemical parameters: Smith and Comb (1991) found that soluble protein content increased to 109 and 120% in pepper seeds primed in -0.90 and -1.35 MPa

Manoimiani, 2011)				
Treatments (%)	Speed of germination	Germination (%)	$DMP (g \text{ seedling}^{-10})$	Vigour index
Unprimed seeds	6.6	83(65.39)	0.351	2603
Hydropriming	8.3	93(74.29)	0.388	3294
Halopriming KNO ₃ 1%	7.5	83(65.40)	0.420	2888
$\mathrm{KNO}_32\%$	8.9	97(81.25)	0.454	3793
KNO_3 3%	8.3	87(68.87)	0.427	2985
$\mathrm{KH}_2\mathrm{PO}_41\%$	8.1	80(63.67)	0.425	2760
$\mathrm{KH}_2\mathrm{PO}_4$ 2%	7.7	83(65.65)	0.433	2794
$ m KH_2PO_4$ 3%	8.3	91(72.21)	0.434	3267
KCl1%	7.9	81(64.15)	0.376	2877
KCl 2%	8.0	87(69.16)	0.384	3089
KCl 3%	7.1	79(62.72)	0.387	2707
NaCl 1%	8.3	88(70.03)	0.389	2947
NaCl 2%	8.1	94(75.43)	0.398	3434
NaCl 3%	6.9	85(66.94)	0.403	2876
Mean	7.9	86(68.94)	0.405	3023
SED	0.175	0.735	0.011	76.665
CD (p = 0.05)	0.349**	1.504**	0.021**	157.04**

Table 1: Influence of halopriming on seed germination and seedling vigour of sunflower hybrid (KBSH 44) seeds (Mohan Kumar and Manoumani, 2011)

NaCl solutions respectively after 12 days of priming and also revealed that there was no significant difference in the soluble protein content between two priming treatments. Davison and Bray (1991) reported that the rate of protein synthesis in 4 day germinated unprimed leek seeds is equivalent to 2 day germinated osmoprimed leek seeds for both embryo and endosperm tissue. They also observed that five polypeptides were found to be synthesized in embryonic tissue of leek seeds after 4 days of priming in a -1.0 MPa polyethylene glycol solution which were not present at fourth day of germination of seeds. Khan (1992) found that two amino acids were incorporated in proteins during the first 24 h of imbibitions of sweet pepper seeds in PEG solutions. In contrast, Dell'Aquila and Spada (1992) revealed that the synthesis of a group of proteins associated with radicle emergence was reduced during imbibition of wheat seeds in NaCl. Fujikura and Karssen (1992) found that cauliflower seeds subjected to controlled deterioration, osmopriming and both, the proteins expression correlated with the rate of germination which was reduced in controlled deterioration and enhanced in osmopriming. Chiu et al. (1995) reported that improvement in germination by priming might be due to enhanced repair of membrane due to reduced leakage of electrolytes in primed watermelon seeds than in control. Job et al. (1997) observed that the priming of sugarbeet seeds with PEG 600 has increased the basic â-subunit of 11-S globulin storage protein correspond to 22 kDa polypeptide in primed sugarbeet seeds when compared with the level seen in unprimed seeds. Bailly et al. (1998) reported that priming of aged sunflower seeds in -2 MPa PEG progressively restored the initial germinability and resulted in marked decrease in the level of conjugated dienes indicating a fall in lipid peroxidation processes. Lin and Sung (2001) found that thermo priming at 20°C increases the activities of isocitrate lyase and malate synthase and these increases in the activities of glyoxysome enzymes which were linked to the improved emergence responses in primed bitter gourd seeds. Gonzalez-Zertuche et al. (2001) observed that the priming of Wigandia urens buried seeds showed increased protein concentration and also induced the synthesis of heat stable proteins of 14 and 23 kDa in buried seeds and proteins of high molecular weight of 43 kDa in primed seeds that were not detected in control seeds.

Cruz-Garcia et al. (2003) revealed that changes in sunflower seeds primed with H_2O_2 diverted a greater part of the cotyledonary resources towards the shoot which was crucial to its earlier establishment and photosynthesis for vigorous growth. Since, this signaling molecule reprogrammed the gene expression leading to *de novo* protein synthesis, a membrane repair mechanism and other substrates available for improved and synchronized germination. Basra et al. (2003b) reported that wheat seeds were subjected to hydropriming for 6, 12 or 24 h and matriconditioned with gunny bags. The best priming treatment was found to be hydropriming for 24 h which registered maximum emergence percentage and lesser Electrical Conductivity (EC) than control. Naglreiter et al. (2005) observed that priming of Pinus sylvestris seeds with PEG+gibberelic acid (200 mg kg⁻¹ of seeds) showed higher free radical content than in unprimed seeds. Whereas seeds primed with K⁺ salts observed only minor changes in the free radical levels. However, both priming treatments showed faster germination rates compared to control without changing the germination percent. Wahid et al. (2008) revealed that priming of sunflower seeds with salicylic acid, H_2O_2 and NaCl induced the *de novo* synthesis of peptides with low molecular weight of 37 and 57 kDa and high molecular weight of 157 and 167 kDa. Mohan Kumar and Manonmani (2011) reported that sunflower hybrid seeds primed with 2% KNO_8 recorded the high α -amylase activity and protease activity during course of germination (Fig. 1 and 2).

Effect of seed priming on molecular parameters: Although priming has been found to improve both the rate and uniformity of germination and emergence in many species, little is known about

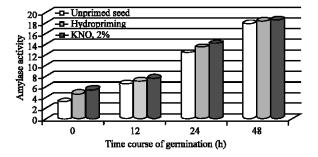


Fig. 1: Influence of seed priming on α-amylase activity (mg maltose min⁻¹) of sunflower hybrid (KBSH 44) seeds during course of germination (Mohan Kumar and Manonmani, 2011)

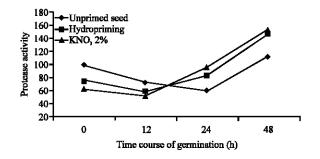


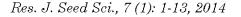
Fig. 2: Influence of seed priming on protease activity (OD value) of sunflower hybrid (KBSH 44) seeds during course of germination (Mohan Kumar and Manonmani, 2011)

the biochemical and molecular mechanisms. A few reported evidence on molecular studies indicated that the increased rate of metabolic process was involved in germination when primed seeds are rehydrated. The beneficial effect of priming has been related to the physiological changes occurring in the partially hydrated embryos and on subsequent germination.

The improved performance of seedling after priming has been explained by the completion of DNA repair mechanisms during the priming period, qualitative and quantitative increase in protein content and rapid declining of reserve materials like phytate and micronutrients during germination of primed seeds (Coolbear and Grierson, 1979; Bray *et al.*, 1989; Job *et al.*, 1997). Dell'Aquila and Taranto (1986) found that the delayed onset of cell division and the low rate of DNA synthesis and its content during osmopriming of wheat seeds were followed by a fast increase of these processes during the early hours of germination in water. They also concluded that in aged seeds, osmoregulation induced more advantageous metabolic changes and enabled this kind of seeds to restore their germinability and vigour more effectively than fresh wheat seeds. Bray *et al.* (1989) revealed that DNA synthesis was detectable in leek embryos during priming in PEG at -1.0 MPa even in the absence of any cell division and upon subsequent germination there was a five fold increase in the rate of DNA synthesis after a 6 to 12 h lag period of germination when compared with the level seen in unprimed seeds.

Coolbear et al. (1990) found that in tomato, var. Moneymaker, there was a large increase in nucleic acid content especially rRNA, within seeds obtained by low temperature presowing treatment and revealed that this was unlikely to be the immediate cause of more rapid seed germination. In contrast, Clarke and James (1991) reported that there was no net increase in RNA and DNA content of leek seeds during osmopriming. Bino et al. (1992) and Lanteri et al. (1993) studied the effects of priming on nuclear replication activity in tomato and pepper seeds by means of flow cytometry. The amount of 4°C nuclei in tomato seed increased to 60% after 21 days of osmopriming treatment. A positive correlation was found between the induction of DNA synthesis measured as the increase in 4°C cells and the efficiency of osmotic treatments to reduce the mean germination time. In leek seeds, Ashraf and Bray (1993) reported that replicative DNA synthesis and nuclear DNA had occurred during osmopriming and germination of leek seeds. Lanteri et al. (1994) revealed that priming of pepper and tomato seeds in -1.1, -1.3 and -1.5 MPa PEG solution for 14 days at 25°C reduced the mean time to germination. An induction of 4°C signals were also found after priming, indicating that during priming the cells of the embryonic root tip had replicated their DNA and arrested at the G_2 phase of the cell cycle. Liu *et al.* (1996) reported that 'fresh PEG priming' of freshly harvested tomato (Lycopersican esculentum Mill) cv. Moneymaker seeds neither alleviated seed dormancy nor promoted DNA replication. However, the addition of $10 \,\mu m \, GA_s$ to the osmotic priming solution triggered the replicative DNA synthesis of fresh priming seeds and further enhanced the germination process.

Gurusinghe *et al.* (1999) reported that radicle tip cells of tomato seeds advanced through the cell cycle during hydrothermal priming resulted in an increase in the percentage of nuclei having 4C DNA content in tomato. Gao *et al.* (2002) reported that germination of *Brassica napus* was enhanced if the seeds were primed either with water for 12 h or with ABA for 40 h with concomitant reduction in transcript level of BnPER1 transcript (peroxiredoxin-antioxidant protein) when compared to control seeds. Hudson *et al.* (2007) found that during seed priming one protease gene (At 5 g 67360, a cucumisin-like serine protease) was significantly expressed and two other protease messages (At 5 g 58870, an FtsH protease and At 4 g 39910, an ubiquitin-specific



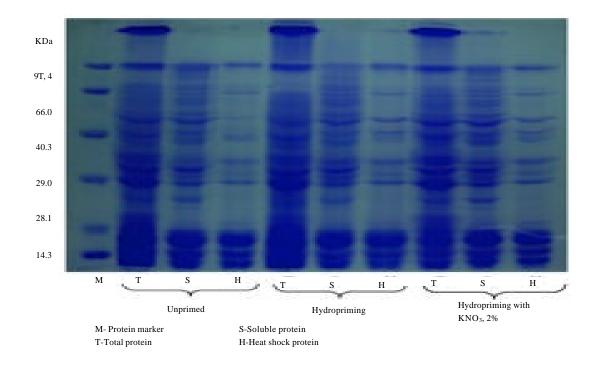


Fig. 3: Protein profiles of sunflower hybrid (KBSH 44) seeds (Mohan Kumar and Manonmani, 2011)

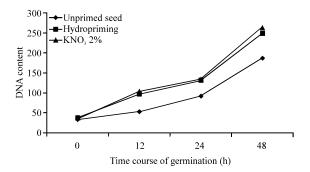


Fig. 4: Influence of seed priming on DNA content (μg g⁻¹) of sunflower hybrid (KBSH 44) seeds during course of germination (Mohan Kumar and Manonmani, 2011)

protease) were significantly repressed. Mohan Kumar and Manonmani (2011) reported that sunflower hybrid seeds primed with 2% KNO_3 recorded the high utilization of proteins and synthesis of new proteins with increased DNA content during course of germination (Fig. 3 and 4).

Effect of seed priming on storability of seeds: Seed storage caused a decrease in the protein content which may be related to oxidation of the amino acids due to the increase in the respiratory activity and advance in the deterioration process of the stored seeds. Thus, prolonged seed storage would increase the metabolic activity of the seeds and consequently decrease the reserve substance content and reduce the dry material weight of the seeds (Bewley and Black, 1982). A number of

studies have indicated that relatively short prehydration treatments (either brief imbibition in water or exposure to high relative humidity) can either improve the tolerance of seeds to subsequent adverse storage condition or improve the vigour of aged seeds (Basu and Pal, 1980; Perl, 1979; Burgass and Powell, 1984; Rao et al., 1987). Argerich and Bradford (1989) reported that primed tomato seeds exhibited delayed germination and a lower mean germination when stored at 30°C for 6 months as compared with the control. Targuis and Bradford (1992) reported that hydropriming of lettuce seeds improved seed germination rate decreased the longevity faster than the nonprimed control seed under controlled deterioration conditions even under mild storage conditions (45°C and 50% relative humidity). Zheng et al. (1994) found that osmopriming of fresh and stored seeds and hydropriming of stored seeds were better than control. Priming induced rapid and uniform germination and rapid emergence of seedlings of canola seeds, which is responsible to repair the age related cellular and subcellular damage of low vigor seeds that may accumulate during seed development (Bray, 1995). Bruggink et al. (1999) reported that the longevity of primed seed was considerably less compared to that of the nonprimed seed lot. Whereas in tomato seeds, a partial restoration of longevity could be obtained by keeping the seeds, after the priming treatment under a mild water and/or temperature stress for a period of several hours to days. Priming temperature influences the success of priming and subsequently affects the longevity of primed seeds (Van Pijlen et al., 1996). The 10°C primed sweetcorn seeds were found to have increased anti-oxidative activity and decreased protein modification and therefore reduced seed deterioration (Murthy and Sun, 2000). Chiu et al. (2002) found that the sweetcorn sh-2 seeds primed at 10°C for 36 h had better storability compared to 20°C-primed seeds when they were stored at 25°C for 12 months. Gurusinghe and Bradford (2001) concluded that osmopriming enhanced the seed germination but longevity of primed seeds in storage was often reduced. However, postpriming heat treatment at 37°C to tomato seeds extended the potential longevity of primed seeds. Basra et al. (2003b) found that canola seeds primed with polyethylene glycol (PEG-10,000) for 4 or 8 h and stored in sealed containers. Among the treatments, osmopriming for 4 h stored seeds resulted more leaf area index, dry matter accumulation, crop growth rate and ultimately higher seed yield than control. Yeh et al. (2005) proved that partial vaccum storage of primed bitter gourd seeds maintain viability upto 12 months. However, primed seeds accumulate more total peroxide than non-primed control after 12 months in non-vaccum storage and this led to a marked decrease in seed longevity. Hill et al. (2007) revealed that primed lettuce seeds stored at 9% moisture content at 38 °C deteriorate faster than primed seeds stored at 6% moisture content at 48°C. Mohan Kumar and Manonmani (2011) reported that haloprimed sunflower seeds with 2% KNO₃ maintain the storage potential by recording maximum germination and field emergence after six months of storage than unprimed seeds (Fig. 5-6).

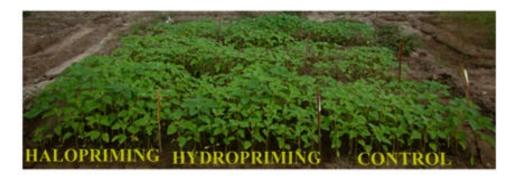


Fig. 5: Effect of seed priming on field emergence potential of sunflower hybrid

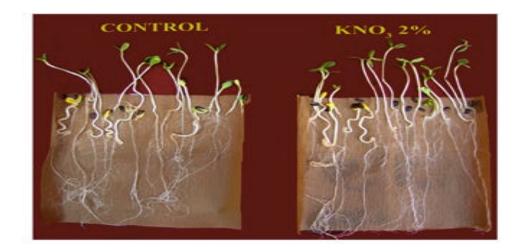


Fig. 6: Effect of seed priming in germination and seedling vigour of sunflower hybrid

CONCLUSION

There is evidence that in most field and horticultural crops have priming led to improvement of germination and seedling establishment. Furthermore, the technique of halopriming having other advantages such as feasibility and low cost. The challenge of technology is to enhance seed performance provides an opportunity for more indepth studies on physiological and biochemical changes that occur during seed treatments. Extending shelf life in storage of primed seeds should be tackled to ensure consistent performance over a wide range of environmental conditions.

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